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# ENTOMON

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# Proteinases in the digestion of an assassin bug *Acanthaspis pedestris* Stål (Hemiptera: Reduviidae)

**Dunston P. Ambrose*, Annie D. Ambrose, A. Dharini and S. Prem Mathi Maran**

*Entomology Research Unit, St. Xavier's College, Palayankottai 627 002, Tamil Nadu, India*
*Email: eruxavier@gmail.com*

ABSTRACT: Trypsin (endopeptidase) and amino peptidase (exopeptidase) activities of anterior and posterior midgut of the assassin bug, *Acanthaspis pedestris* Stål observed at various time intervals after the commencement of feeding suggest the following model of digestive organization. The protein and amino acids in the liquefied preorally digested food is largely digested by anterior midgut enzymes as evidenced by its robust trypsin as well as amino peptidase activities. The enzymes of posterior midgut particularly amino peptidases are involved in the digestion of amino acids prior to absorption. On prey deprivation, trypsin activity depleted both in the anterior and posterior midgut while amino peptidase activity depleted in the anterior midgut and increased in the posterior midgut. 

KEYWORDS: *Acanthaspis pedestris*, assassin bug, digestive organization, prey deprivation, proteinases

## INTRODUCTION

Proteinases viz., endopeptidases and exopeptidases are the most important liquefaction enzymes in predators (Cohen, 1993, 2000). The salivary secretion and digestive fluids of many heteropteran predators including assassin bugs contain trypsin and amino peptidase (Cohen, 1990, 1993). The endopeptidases liquefy and mobilize the proteinaceous structures in the prey (Cohen, 1989, 1995). The ingested food undergoes further hydrolysis within the anterior midgut by endopeptidases and subsequently by exopeptidases in the posterior midgut. The interplay of morphological, behavioural and biochemical adaptations in the digestive process of predators promote efficient food utilization and also promote conversion ratio (Cohen, 1993). However, no such knowledge is available for any heteropteran from India. Hence, an attempt was made to understand the activities of proteinases in anterior and posterior midgut of a reduviine

*Corresponding author

assassin bug, *Acanthaspis pedestris* Stål, a predator on various insect pests in India (Ambrose, 1999). This knowledge may help in developing a suitable artificial diet for mass culturing of the predator for use in IPM technologies (Ambrose, 2009).

## MATERIALS AND METHODS

*Acanthaspis pedestris* were reared in the laboratory under 30–35 °C; 75–85% RH and 11–13 h photoperiod on *Mylabris pustulata*. Ten day old bugs were used for the experiments. The weights of predators and prey chosen ranged between 175 to 185 mg and 240 to 250 mg, respectively.

The trypsin and amino peptidase activities of fed *A. pedestris* at different time intervals and prey deprived predators were studied as follows. (1) anterior midgut and posterior midgut assayed at 0.25, 0.5, 1.0, 1.5, 2.0, 2.75, 3.0, 3.5, 4.0, 4.5, 12 and 24 h from the commencement of feeding i.e., from the moment the predator inserted rostrum and started sucking, and (2) anterior midgut and posterior midgut assayed from 0, 1, 2, 4, 6 and 8 day prey deprived *A. pedestris*. The homogenates of anterior midgut and posterior midgut of predators were prepared following the methodology of Cohen (1993). The presence of proteinases was determined by the casein-gel method (Bjerrum, 1975) and confirmed by using haemoglobin as the substrate. Then samples were assayed with two artificial protein substrates viz., benzoyl-DL arginine-p-nitroanilide (BapNA) and leucine-p-nitroanilide (LpNA) (E. Merck, 64271, Darmstadt, Germany) to determine the nature of the proteinases i.e., endopeptidase (trypsin) and exopeptidase (amino peptidase) (Cohen, 1993). One enzyme unit was estimated as the amount of material required to hydrolyse 1 $\mu$M of substrate/min. Micromoles of substrate hydrolysed were determined using the following extinction coefficients of substrates: BapNA and LpNA $\Delta_{\Sigma}410 = 8800$ (Erlanger *et al.*, 1961). The concentrations of protein in samples were measured using bovine serum albumin (BSA) as standard (Lowry *et al.*, 1951). Six replicates were maintained in each observation. The data were subjected to relevant statistical analysis.

## RESULTS AND DISCUSSION

The trypsin activity of *A. pedestris* in the anterior and posterior midgut remained low with slow increase up to 1.5 h after the commencement of feeding (Table 1). At 2.0 h the trypsin activity suddenly increased (from 1.39 to 6.28) and reached its peak (9.95) at 2.75 h. Though the trypsin activity started declining thereafter, it remained at a moderately high level up to 4.5 h (5.65). The hike in trypsin activity recorded only after a period of 1.5 h after the commencement of feeding reveals that the trypsin recorded is secreted by the anterior midgut itself and not carried from the salivary gland secretion. In the posterior midgut also peak activity of trypsin is found only 2.0 h after the commencement of feeding as observed in the anterior midgut and it remained high up to 3.0 h. Since the trypsin activities from the anterior midgut and the posterior midgut coincided it indicates that either the trypsin from the anterior midgut may be reaching the posterior midgut or it may be secreted by the posterior

midgut itself. But the relatively low level of trypsin activity in the posterior midgut when compared to that of anterior midgut suggests that it may be originating from the anterior midgut and carried over to the posterior midgut.

The amino peptidase activity of *A. pedestris* commenced in the anterior midgut at 2.0 h (4.27) and continuously increased up to the end of the observation i.e., 24 h (17.13) (Table 1). Though amino peptidase activity in the posterior midgut also commenced at 2.0 h (4.35) as in the anterior midgut, it was comparatively lower up to 4.0 h after the commencement of feeding. However, the amino peptidase activity in the posterior midgut thereafter became higher (16.24 to 24.75) than in the anterior midgut (15.17 to 17.13). Such differential activities of trypsin and amino peptidase in the midgut at varied time intervals after the commencement of feeding were observed in haematophagous insects such as *Stomoxys calcitrans* (L.) (Diptera) and *Rhodnius prolixus* Stål (Hemiptera) (Houseman *et al.*, 1985; Schneider *et al.*, 1987) as well as the entomophagous reduviids, *Zelus renardii* Kolenati (Cohen, 1993) and *Rhynocoris marginatus* (Fabricius) (Ambrose and Maran, 1999, 2000).

In prey deprived insects, within a day the trypsin and amino peptidase activity got drastically reduced in both anterior and posterior midgut (Table 1). Though amino peptidase activity of anterior midgut decreased (from 27.64 to 8.11) as observed for trypsin activity, it increased (from 32.5 to 43.36) in the posterior midgut. A similar activity of amino peptidase was observed by Cohen (1993) in *Z. renardii* and Ambrose and Maran (1999, 2000)) in *R. marginatus*. The decrease in endopeptidase trypsin activity and increase in exopeptidase amino peptidase activity in the midgut of prey deprived *A. pedestris* could be attributed to the reason that the trypsin activity coincided to the protein content whereas such correlation did not exist between the amino peptidase activity and midgut protein. Trypsin activity is controlled by secretagogue mechanism but amino peptidase is not controlled by it as observed in two haematophagous insects *S. calcitrans* and *R. prolixus* (Houseman *et al.*, 1985).

The observation on the trypsin and amino peptidase activities of anterior and posterior midguts suggests the following model of digestive organization for *A. pedestris*. The protein and amino acids in the liquefied preorally digested food is largely digested by the anterior midgut enzymes as evidenced by its robust trypsin as well as amino peptidase activities. The enzymes of posterior midgut particularly the amino peptidase also involve in digestion prior to absorption. They contribute more to the hydrolysis of amino acids as observed in two harpactorine assassin bugs *Z. renardii* (Cohen, 1993) and *R. marginatus* (Ambrose and Maran, 1999, 2000).

The robust trypsin and amino peptidase activities in the anterior midgut and amino peptidase activity in the posterior midgut facilitate rapid hydrolysis of protein and digestion of prey and enabled to accelerate the gut filling and gut emptying regimen of *A. pedestris* as observed in *Z. renardii* (Cohen, 1993). Any such acceleration of food processing would impact the effectiveness and fitness of predators in terms of number and size of prey that they can consume (Bailey, 1986), an index to choose a biological control agent.

TABLE 1. Trypsin and amino peptidase activities in anterior midgut (AMG) and posterior midgut (PMG) of *Acanthaspis pedestris* at various time intervals after commencement of feeding and prey deprivation

| Period after the commencement of feeding (h) | Trypsin activity ($\mu$M BApNA hydrolysed/min/mg protein) | | Amino peptidase activity ($\mu$M LPNA hydrolysed/min/mg protein) | |
|---|---|---|---|---|
| | AMG | PMG | AMG | PMG |
| 0.25 | $0.38^{1,a}$ | $0.26^{1,a}$ | $0.62^{1,a}$ | $0.48^{1,a}$ |
| 0.50 | $0.53^{2,a}$ | $0.27^{2,b}$ | $1.26^{2,a}$ | $0.62^{2,b}$ |
| 1.00 | $0.81^{3,a}$ | $0.73^{3,a}$ | $1.76^{3,a}$ | $1.02^{3,b}$ |
| 1.50 | $1.39^{4,a}$ | $1.56^{4,a}$ | $2.72^{4,a}$ | $3.41^{4,b}$ |
| 2.00 | $6.28^{5,a}$ | $3.65^{5,b}$ | $4.27^{5,a}$ | $4.35^{5,a}$ |
| 2.50 | $7.11^{6,a}$ | $5.36^{6,b}$ | $6.68^{6,a}$ | $6.24^{6,a}$ |
| 2.75 | $9.95^{1,a}$ | $4.25^{1,b}$ | $8.73^{1,a}$ | $4.24^{1,b}$ |
| 3.0 | $8.71^{2,a}$ | $3.70^{2,b}$ | $9.39^{2,a}$ | $5.14^{2,b}$ |
| 3.5 | $8.43^{2,a}$ | $3.17^{2,b}$ | $11.91^{3,a}$ | $7.42^{3,b}$ |
| 4.0 | $6.78^{4,a}$ | $1.95^{3,b}$ | $13.87^{4,a}$ | $10.32^{4,b}$ |
| 4.5 | $5.65^{5,a}$ | $1.63^{3,b}$ | $15.17^{5,a}$ | $16.24^{5,b}$ |
| 12.0 | $2.76^{6,a}$ | $1.22^{4,b}$ | $16.90^{6,a}$ | $21.06^{6,b}$ |
| 24.0 | $1.56^{7,a}$ | $0.80^{5,b}$ | $17.13^{7,a}$ | $24.75^{7,b}$ |
| Days of prey deprivation | | | | |
| 0 | $2.79^{1,a}$ | $1.19^{1,b}$ | $27.64^{1,a}$ | $32.50^{1,b}$ |
| 1 | $1.50^{2,a}$ | $0.85^{1,b}$ | $15.45^{2,a}$ | $34.26^{1,b}$ |
| 2 | $1.15^{3,a}$ | $0.58^{2,b}$ | $11.70^{3,a}$ | $36.78^{1,b}$ |
| 4 | $1.06^{3,a}$ | $0.50^{2,b}$ | $10.89^{3,a}$ | $43.2^{2,b}$ |
| 6 | $0.71^{4,a}$ | $0.36^{3,b}$ | $8.80^{4,a}$ | $44.14^{2,b}$ |
| 8 | $0.46^{5,a}$ | $0.26^{3,b}$ | $8.11^{4,a}$ | $43.36^{2,b}$ |

$n = 6$.

Values superscribed with different numerals and alphabets indicate significant differences between time intervals (between rows) and anterior and posterior midgut (between columns) respectively at $p = 0.001$.

The digestive organization of *A. pedestris* also enables it to abandon a partially consumed prey and predating upon another prey, a desirable biocontrol characteristic feature (Ambrose, 1999). Such partial consumption of prey is also influenced by physiological constraints such as gut capacity and/or digestive rate limitations that in turn influence the functional response of predators (Hassell, 1978; Ambrose, 1999).

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# Purification and partial characterization of a male derived peptide that enhances egg maturation and oviposition in female cotton bollworm, *Helicoverpa armigera* (Lepidoptera: Noctuidae)

**Shobha Anand, Geetha Bali and Manjulakumari Doddamane***

*Department of Microbiology and Biotechnology, Bangalore University, Bangalore 560 056, Karnataka, India*
*Email: shobhaanand01@gmail.com*
*Email: baliccet@vsnl.com*
*Email: manjulakumari_doddamane@yahoo.co.in*

ABSTRACT: The present study was planned to isolate male factors that are involved in regulating the physiology of egg maturation and oviposition in *Helicoverpa armigera*. The active factors present in combined accessory glands and ejaculatory duct duplex were extracted in acidic conditions and purified by using weak–cation exchange chromatography and reverse phase HPLC. The bioactivity during each purification step was determined by injecting the factors into the body cavity of virgin day-2 female. Mass spectrometric analysis was performed to determine the molecular weight (MW) and sequence of the active factor. Using these procedures a peptide that enhanced egg laying and egg maturation was isolated and purified. The peptide was found to be thermostable and 6.7 kDa in MW with a probable pI of 5.2. Subsequent Trypsinisation and sequence analysis yielded a partial sequence rich in leucine/isoleucine amino acid residues. 

KEYWORDS: egg maturation, fecundity enhancing substance, *Helicoverpa armigera*, oviposition, peptide purification

## INTRODUCTION

In females of *Helicoverpa armigera* (Lepidoptera: Noctuidae) moth, both reproductive potential (egg maturation) and output (oviposition) are enhanced not only by mating, but also by proteinaceous factors derived from male accessory glands (Acg) and ejaculatory duct duplex (Ed) (Manjulakumari *et al.*, 2009). On SDS-PAGE analysis of these tissues, it was observed that a number of proteins ranging from 116 kDa to 2 kDa are present and the protein profile of Acg was similar to that of Ed (Shobha,

*Corresponding author

2008), indicating that common factor/s could be regulating both egg maturation and oviposition. In the present studies we describe the steps involved in purification and characterization of a factor derived from combined Acg-Ed tissues that regulate not only egg maturation but also oviposition in *H. armigera*.

## MATERIALS AND METHODS

Maintenance of *H. armigera* moths, dissection of Acg and Ed, and extraction of proteinaceous factors were as described previously (Manjulakumari *et al.*, 2009).

### Extraction and purification of the active factor

The proteinaceous factors from combined Acg-Ed were purified as described by Kingan *et al.* (1993) with slight modification. Approximately 300 pairs of Acg-Ed tissues were used. After extraction, desalted Acg-Ed sample was passed through a CM Sep-Pak cartridge (Waters, USA) and rinsed successively with 0 mM, 200 mM and 1 M sodium chloride (NaCl) dissolved in 10 mM ammonium acetate and 20% acetonitrile (AcN) solution. The four rinses including the first rinse containing unretained material were collected separately, desalted and tested for bioactivity. The bioactive fractions were further fractionated by reverse phase HPLC (RP-HPLC). Fractionation was performed in a wide-pore C4 column (Vydac, 4.6 mm × 25 cm, The Separations Group, Hesperia, CA) using a linear gradient of 10–70% AcN in 0.1% trifluoroacetic acid for 90 min at a flow rate of 1 ml/min. Further purification of bioactive fractions was performed using a C-18 reverse-phase column (Vydac, 4.6 mm × 25 cm, The Separations Group, Hesperia, CA) and eluted with a linear gradient as described above. Elution was monitored at 280 nm, and the peaks were collected manually to ensure optimal separation. The concentration of protein during each step in purification was determined by Lowry *et al.* (1951) method.

### Biochemical characterization of the active factor

Purity, molecular weight (MW) and partial sequence of the bioactive peptide was determined by Mass Spectrometry (MS) at the National Centre for Biological Sciences Mass Spec facility, Bangalore, India. In-solution trypsin digestion of active factor was carried out by Kinter and Sherman method (2000). Isoelectrofocusing (IEF) was performed on a 1 mm thick 15% polyacrylamide gel (Sambrook *et al.*, 1989) to determine the apparent pI value of the purified peptide using Pharmalyte 3–10. After focusing, the proteins on the gel were fixed with 10% trichloroacetic acid solution. Thermostability of the HPLC purified peptide was checked by treating the females with peptide fraction that was incubated in a boiling water bath for 10 min.

### Determination of bioactivity of the isolated factor/s

Activity of the isolated factors after each purification step was determined by bioassays standardized in our laboratory. The bioassay experiments consisted of two groups

of 30 day-2 virgin females. The first group consisted of females injected with partially purified factors/purified factors (2 pairs of Acg-Ed equivalent) and the second group contained females injected with saline (3 $\mu$l in volume). Egg maturation and oviposition activity was recorded and oviposition index (O.I.) calculated as described previously (Manjulakumari *et al.*, 2009).

Normal pattern of oviposition and egg maturation was observed in day-2 mated and age matched virgin females and these moths served as untreated controls.

Each experiment was replicated thrice. Data were subjected to ANOVA and Student-Newman-Keuls test for comparisons of means.

## RESULTS

### Pattern of oviposition and egg maturation in mated and virgin moths

General observations revealed that moths neither mate nor lay eggs on the day of emergence. All the results presented are from day-2 mated moths and day-2 virgins. The time interval that is depicted in Tables 1–4 and Figs. 1–2 is starting from day 2.

On day 2, both mated and virgin females lay very few eggs although the number of eggs produced by mated moths was 221 ± 41. During the observation period of six days oviposition attained a peak on day 3 in the mated moths while in virgin females the oviposition did not show any regular pattern. It was also observed that the number of eggs laid (Table 1) or the number of eggs matured (Table 2) in mated moths was always more than those laid by virgin females on all the days.

### Effect of Acg-Ed factors on oviposition and egg maturation

Females injected with desalted crude accessory gland material homogenized using Bennett's buffer laid (968 ± 62) 2.99 times more eggs compared with the moths receiving saline (323 ± 80). By the end of observation period, the number of eggs matured was 2.01 times more in experimental moths (1122 ± 76) when compared to controls (556 ± 127) and was statistically significant ($p < 0.001$). After further purification in weak cation–exchange cartridge, the oviposition enhancing and egg maturation activity was retained in the cartridge when applied in the absence of NaCl (Fraction 1 and 2) and could be eluted with buffer containing 200 mM NaCl (Fraction 3) and 1M NaCl (Fraction 4) (Figs. 1–2).

### RP-HPLC fractionation

The active fractions that eluted out in the cation exchange cartridge (Fraction 3 and 4) were further fractionated separately by RP-HPLC using a C4 column. Initially, the samples were pooled from ten adjacent 1 min fractions beginning with min 5 for bioassays. In both fractions 3 and 4, the bioactivity was found in pool of fractions eluted between 45–55 min. When individual fractions in and around this zone were assayed, it was found that the peak eluted between 46.4–47.9 min in Fraction 3, and peak eluted between 45–47 min in Fraction 4 stimulated egg

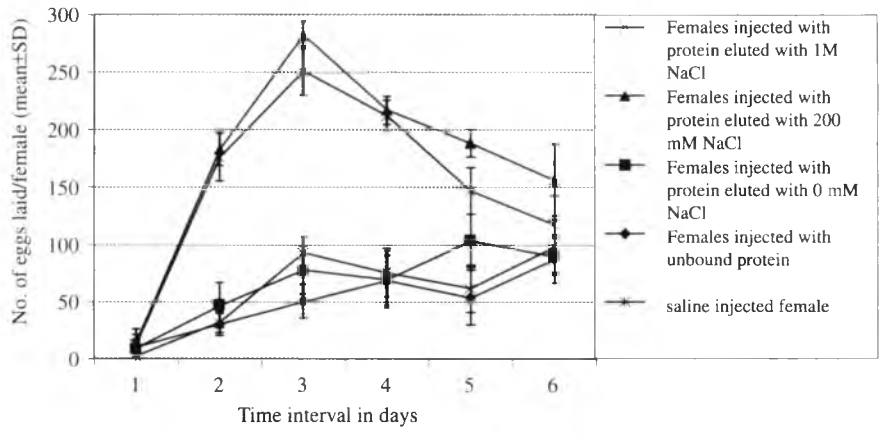


FIGURE 1. No. of eggs laid by females treated with cation exchange purified Acg-Ed fractions in *H. armigera* for six days following treatment.

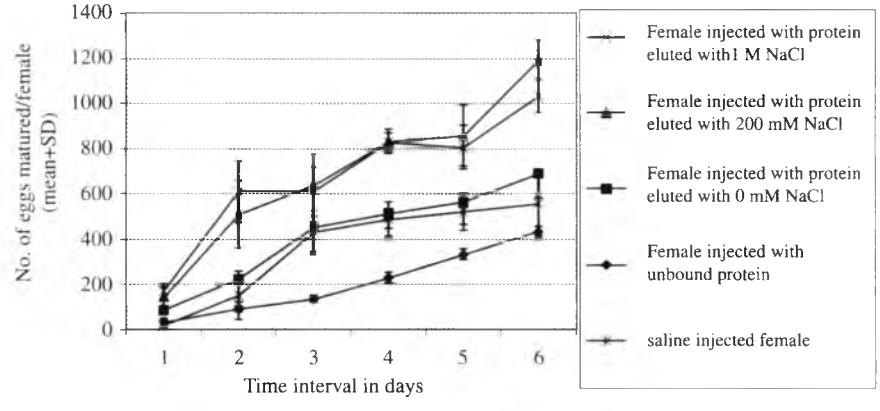


FIGURE 2. No. of eggs matured by females treated with cation exchange,purified Acg-Ed fractions in *H. armigera* for six days following treatment.

maturation and oviposition, presenting evidence that the bioactive factors obtained from either Fraction 3 or Fraction 4 had similar chromatographic properties. Hence peak fractions showing similar bioactivity in Fraction 3 and Fraction 4 were pooled and rechromatographed using C18 column. A single peak eluted between 47.33–48.5 min (Fig. 3). The concentration of 47.33–48.5 min peak fraction obtained from approx. 300 Acg-Ed was 874.6 $\mu$g.

### Determination of bioactivity of HPLC-peak fraction

For bioassay experiments each female was treated with approx 2-Acg-Ed equivalent i.e., 3 $\mu$g of the purified RP-HPLC peak fraction dissolved in 3 $\mu$l saline. The females

TABLE 1. No. of eggs laid by untreated virgin and mated *H. armigera* for six days

| Treatment | Days after treatment | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Virgin females | $4 \pm 2^{a}$ | $17 \pm 7^{a}$ | $52 \pm 19^{b}$ | $39 \pm 12^{b}$ | $109 \pm 27^{c}$ | $105 \pm 12^{c}$ |
| Mated females | $7 \pm 3^{a}$ | $219 \pm 13^{d}$ | $266 \pm 32^{d}$ | $243 \pm 44^{d}$ | $216 \pm 36^{d}$ | $188 \pm 28^{d}$ |

$N = 6$ for all groups.
Means followed by different letters in both rows and columns are significantly different at $p < 0.05$.

TABLE 2. No. of eggs matured by untreated virgin and mated *H. armigera* for six days

| Treatment | Days after treatment | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Virgin females | $12 \pm 4^{a}$ | $34 \pm 12^{b}$ | $70 \pm 35^{c}$ | $301 \pm 57^{d}$ | $499 \pm 82^{e}$ | $621 \pm 80^{e}$ |
| Mated females | $234 \pm 41^{d}$ | $444 \pm 100^{e}$ | $551 \pm 160^{e}$ | $805 \pm 136^{f}$ | $951 \pm 121^{f}$ | $1232 \pm 62^{g}$ |

$N = 6$ for all groups.
Means followed by different letters in both rows and columns are significantly different at $p < 0.05$.

TABLE 3. Number of eggs laid by *H. armigera* females following treatment with purified peptide

| Treatment | Days after treatment | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Females treated with FES | $10 \pm 3^{a}$ | $250 \pm 47^{e}$ | $315 \pm 15^{e}$ | $298 \pm 40^{e}$ | $132 \pm 23^{d}$ | $82 \pm 27^{c}$ |
| Females treated with FES after boiling | $14 \pm 4^{a}$ | $211 \pm 45^{d}$ | $289 \pm 10^{e}$ | $262 \pm 33^{e}$ | $182 \pm 45^{d}$ | $126 \pm 41^{d}$ |
| Females treated with saline (Control) | $2 \pm 2^{a}$ | $32 \pm 12^{b}$ | $92 \pm 4^{c}$ | $75 \pm 29^{c}$ | $61 \pm 12^{c}$ | $98 \pm 23^{c}$ |

$N = 6$ for all groups.
Means followed by different letters in both rows and columns are significantly different at $p < 0.05$.

treated with this peak fraction showed oviposition (Table 3) and egg maturation (Table 4) enhancing activity and was named as fecundity enhancing substance (FES) to indicate that it contains oviposition and egg maturation enhancing factors. It was also seen that the females treated with FES after subjecting it to boiling, oviposit and mature eggs at a rate similar to FES treated moths (Tables 3–4).

Though mated moths laid the highest number of eggs at any given time compared with females in the other treatment groups. O.I. of moths treated with Bennett's buffer

TABLE 4. Number of eggs matured by *H. armigera* females following treatment with purified peptide

| Treatment | Days after treatment | | | | | |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| Females treated with FES | $224 \pm 61^{c}$ | $425 \pm 71^{e}$ | $614 \pm 39^{f}$ | $751 \pm 82^{g}$ | $899 \pm 58^{h}$ | $1245 \pm 89^{i}$ |
| Females treated with FES after boiling | $214 \pm 44^{c}$ | $366 \pm 70^{d}$ | $547 \pm 68^{e}$ | $776 \pm 56^{g}$ | $945 \pm 60^{h}$ | $1189 \pm 45^{i}$ |
| Females treated with saline (Control) | $89 \pm 9^{a}$ | $194 \pm 24^{b}$ | $259 \pm 40^{c}$ | $375 \pm 20^{d}$ | $457 \pm 46^{e}$ | $667 \pm 61^{f}$ |

$N = 6$ for all groups.
Means followed by different letters in both rows and columns are significantly different at $p < 0.05$.

TABLE 5. Oviposition index of the *H. armigera* treated and untreated females

| Treatment Group | Oviposition Index (%) |
|---|---|
| Virgin female | 52 |
| Mated female | 92 |
| Saline injected female | 58[a] |
| Females injected with crude Acg-Ed acidic extracts | 86[b] |
| Females injected with unbound protein | 69[a] |
| Females injected with protein eluted with 0 mM NaCl | 58[a] |
| Females injected with protein eluted with 200 mM NaCl | 88[b] |
| Females injected with protein eluted with 1M NaCl | 89[b] |
| Females injected with FES | 87[b] |
| Females injected with FES after boiling | 91[b] |

[a] Treated females with Oviposition index similar to virgin females.
[b] Treated Females with oviposition index similar to Mated females.

extract (86%), FES (87%) or FES after subjecting to boiling treatment (91%) was comparable to that of mated moths (92%) clearly indicating that the effect of the factor was not reduced significantly ($p > 0.05$) during purification. O.I. of the saline treated controls (58%) was comparable to that of untreated virgin females (52%) (Table 5).

## Biochemical characteristics

As determined by TOF MS ES+, FES had a MW of 6720.34 ± 0.06 Da and was about 98% pure. The IEF profile indicated that FES had a pI value of about 5.2. Trypsinisation of FES and subsequent analysis yielded a part of the sequence (Glu-Asn-Gly-Glu-Leu/Ile-Val-Asp-Leu/Ile-Leu/Ile).

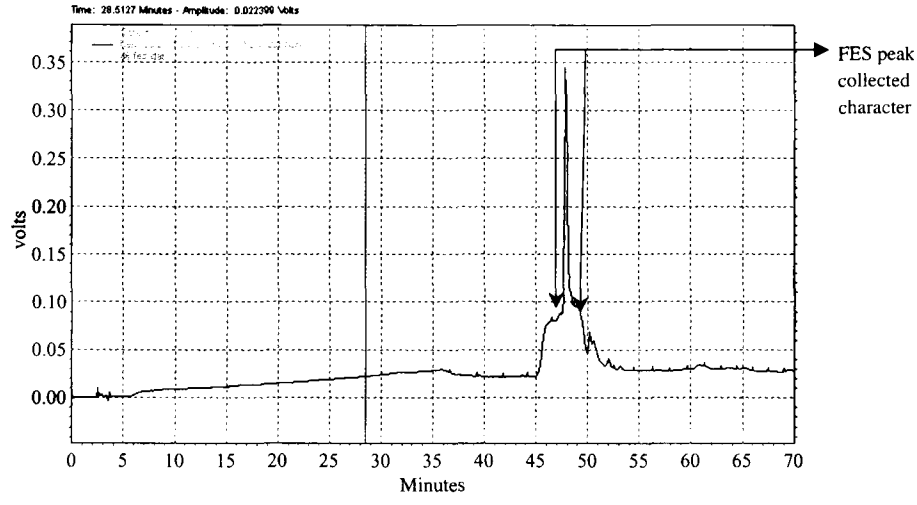


FIGURE 3. Elution profile of fecundity enhancing substance (FES) isolated from MAG of *H. armigera* using C18 column

## DISCUSSION

The increased fecundity that results from mating in many species is due to the transfer of regulating factors derived from male reproductive tissues (as reviewed by Gillott (2003); Kubli (1992); Wolfner (1997)). Our study also confirms this view. Injection of desalted crude extracts/ partially purified extracts/purified factor derived from Acg-Ed stimulated an increase in the rate of oviposition and egg maturation in virgin females. We are yet to understand the mechanism by which FES regulates these processes, but apparently, the stimulus of egg maturation and oviposition is blood-borne, since injection into the body cavity induced these changes.

There are only few reports describing the purification and characterization of oviposition and egg maturation stimulating factors because of the difficulty in sequencing the pure fraction. Till date, *D. melanogaster* sex-peptide (Kubli, 2003) and 30 KDa protein in *Melanoplus sanguinipes* (Yi and Gillott, 1999) are the only well characterized male factors.

After initial extraction in the present studies, mass spectrophotometric analysis showed the presence of low MW protein/peptides in Acg-Ed. But Jin and Gong (2001) were able to isolate many high MW proteins ranging from 14.4–205 KDa from Acg in *H. armigera* following similar methodology. Even on repeating our procedures we were not able to replicate their results. Further, mass spectrophotometric analysis of the purified fraction confirmed that the active factor in the present studies was a low MW peptide (6720.34 $\pm$ 0.06 Da). Kingan *et al.* (1993) have also observed the presence of peptides between 2 KDa and 12 KDa in molecular mass in *H. zea* as the highly acidic Bennett's buffer is known to precipitate high MW factors. For extraction of small MW peptides like allatostatins from *Aedes aegypti* (Veenstra *et*

*al.*, 1997), HezPSP (Kingan *et al.*, 1995), OSS from *H.zea* (Bali *et al.*, 1996) similar methodology was followed. The pI (5.2) and the retention profile of the active factor during purification reflected the presence of high proportion of acidic and hydrophobic amino acids. Leucine/Isoleucine was found to be the major hydrophobic component on preliminary analysis of the amino acid composition which was confirmed by further sequence analysis.

Our initial attempts to sequence the FES by automated Edman degradation yielded no signal in the first cycle indicating N-terminal blockage. Upon trypsinisation of FES and subsequent analysis, a part of the sequence was obtained. At present further enzyme digestion and analysis is in progress. Although novelty of this peptide cannot be ascertained with these results, we are sure that FES is not similar to HezPSP. We were able to elute a HPLC peak with HezPSP-like sequence similarity between 49.4–51.9 min just after eluting FES peak in the C4 HPLC column. On further purification in C18 column and analysis, this fraction was found to have a MW of 7 kDa. When injected into the virgin females, FES-like activity was not observed but the females did not call nor mate on the day of treatment (results to be published).

Studies have shown that several components of apparently similar function are present in the reproductive system. In *D. suzukii* and *D. biarmipes*, there are indications that more than one regulating molecule may exist. In *D. suzukii* the oviposition stimulating substance (OSS) isolated by Ohashi *et al.* (1991) has a different amino acid sequence from those of the two peptides characterized by Schmidt *et al.* (1993). In *D. biarmipes* (Imamura *et al.*, 1998) the OSS isolated from Acg-Ed was different from OSS isolated from ejaculatory duct. Our studies also indicate that there may be more than one FES in *H. armigera* or that the 6.7 kDa MW peptide obtained in our studies is only a part of the larger protein of 55–66 kDa in MW that was isolated in earlier studies (Jin and Gong, 2001). These findings suggest that there is either redundancy in the reproductive system or that these factors regulating reproduction are fast evolving. The targets of these proteins with similar function may also be different.

It is interesting to note that purified FES did not loose its biological activity on boiling. Our hypothesis is that once the amino acid sequence of this peptide is identified, and the mode of action established, it would be possible to employ this heat stable peptide either by synthesizing it or its analogues or through gene expression to influence the behavior and physiology of the insect.

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# Diversity, spatio-temporal distribution and biting activity of mosquitoes in Tripura State, India

**Sunil Dhiman*, Reji Gopalakrishnan, Diganta Goswami, N. G. Das, Indra Baruah, Bipul Rabha, P. K. Talukdar and Lokendra Singh**

*Defence Research Laboratory, Tezpur, Assam 784 001, India*
*Email: dhiman_81@indiatimes.com*

ABSTRACT: The State of Tripura has a hot and humid climate which is favourable for the incidence and spread of mosquito borne diseases. Studies were carried out on the distribution of mosquito species and their biting activity with emphasis on disease vectors in the State during 2006–08. Adult mosquitoes were collected through indoor resting collections and CDC light traps from 78 sites. Twenty nine species of mosquitoes belonging to six genera, *Anopheles* (17), *Culex* (6), *Mansonia* (3), *Coquillettidia* (1), *Armigeres* (1) and *Aedes* (1) were recorded, which included vectors of malaria, Japanese encephalitis, filariasis and dengue. The malaria vector, *Anopheles minimus* and filariasis vector *Culex quinquefasciatus* were found to be widely distributed in the region. Other malaria vectors recorded from the region include *An. dirus*, *An. fluviatilis* and *An. philippinensis/nivipes*. *Mansonia annulifera*, the principal vector of Malayan filariasis and *Aedes albopictus*, vector of dengue were also recorded from the state. *An. barbirostris*, *An. vagus*, *Cx. malayi*, *Cx. vishnui*, *Armigeres subalbatus* and *Ma. uniformis* were found to be prevalent in all seasons.


KEYWORDS: Tripura, mosquito, vector, distribution, biting activity

## INTRODUCTION

The State of Tripura, in the northeastern region of India, is situated between 22°56′N and 24°31′N latitude and 91°10′E and 92°21′E longitude. Its hot and humid climate is conducive for incidence and spread of vector borne diseases. Malaria, spread by anopheline mosquitoes, is the most important vector borne disease in this part of the country. The hilly and undulating terrain, long international border, unstable population and migration of mosquitoes across the border make disease management a daunting task (Malhotra, 2000; Dev *et al.*, 2003). One of the essential components of malaria control programme is mosquito control, the success of which relies on

*Corresponding author

the knowledge of the species of vector mosquitoes and their bionomics. Fifty eight species of *Anopheles* have been recorded in India, out of which six act as the main malaria vectors. These are *An. culicifacies*, *An. dirus*, *An. fluviatilis*, *An. minimus*, *An. sundaicus* and *An. stephensi*. Apart from them, *An. philippinensis/nivipes*, *An. varuna*, *An. annularis* and *An. jeyporiensis* are considered to be vectors of local importance.

Survey of mosquito diversity and their distribution in Tripura is a prerequisite for attempts to control malaria and other mosquito borne diseases in the region. Mosquito surveys in Tripura have been carried out earlier by Misra and Dhar (1955), Das *et al.* (1991) and Prakash *et al.* (1998). The present study was aimed at updating information on the diversity, distribution and seasonal prevalence of mosquito species in Tripura State with special reference to disease vectors.

## MATERIALS AND METHODS

Mosquito surveys were conducted during 2006–08 in the South Tripura and the North Tripura districts (referred to as the South and the North henceforth) of Tripura State. Forty seven sites in the South and 31 in the North were randomly selected for the study. Each site was surveyed four times — twice during the pre-monsoon (March–April) and twice during the monsoon (May–September) seasons in consecutive years. Adult mosquitoes were collected using battery-operated CDC miniature light traps during 1800–0600 h from human dwellings and cattle sheds. Indoor resting collections were also made from the same sites using aspirators and flashlights during 0500–0700 h. The adult mosquitoes were identified using the keys of Barraud (1934), Wattal and Kalra (1961) and Nagpal *et al.* (2005).

It is very difficult to differentiate the species *An. philippinensis* and *An. nivipes* based on adult morphology (Prakash *et al.*, 2006). Proper identification is possible only on the basis of larval and pupal characters (Reid, 1967). Since the present study was based on adults obtained through light trap collections, these two species could not be reliably separated. Hence they are denoted as *An. philippinensis/nivipes*. The percent distribution of each species was determined as the number of sites from which the species was recorded $\times 100$/total number of collection sites. The species recorded were classified based on percent distribution as sparse (<33%), moderately distributed (33–66%) and widely distributed (>66%) (Shetty *et al.*, 2007).

The biting activity of mosquitoes was studied by recording human landing catches during 1800–0600 h from five sites each from the two districts. Informed consent was obtained from the volunteers for the study. The collectors were equipped with flashlights, aspirators and timers. The mosquitoes alighting on the lower legs of the human baits were caught and the number of landings per man per hour was determined.

## RESULTS AND DISCUSSION

A total of 29 mosquito species were recorded in the South and the North districts of Tripura State (Table 1). In the South, 27 species were recorded of which 17 belonged

TABLE 1. Spatial distribution of mosquito species in the South and the North districts of Tripura State (2006–08)

| Mosquito species | South Tripura | | | North Tripura | | | Total | | |
|---|---|---|---|---|---|---|---|---|---|
| | Adult collection (%) | | Distribution | Adult collection (%) | | Distribution | Adult collection (%) | | Distribution |
| | IR | LT | (%) | IR | LT | (%) | IR | LT | (%) |
| *An. aconitus* | 0.03 | 0.09 | 25.53 | 1.37 | 1.06 | 51.61 | 0.35 | 0.21 | 35.90 |
| *An. annularis* | – | 0.02 | 12.77 | – | – | – | – | 0.02 | 7.69 |
| *An. barbirostris* | 15.45 | 6.33 | 74.47 | 0.80 | 1.41 | 54.84 | 12.02 | 5.75 | 66.67 |
| *An. crawfordi* | 12.67 | 7.68 | 100 | 0.68 | 0.42 | 16.13 | 9.87 | 6.82 | 66.67 |
| *An. culicifacies* | – | 0.19 | 12.77 | – | – | – | – | 0.17 | 7.69 |
| *An. dirus* | 0.10 | 0.03 | 12.77 | 0.46 | 0.28 | 29.03 | 0.19 | 0.06 | 19.23 |
| *An. fluviatilis* | – | 0.04 | 12.77 | – | – | – | – | 0.03 | 7.69 |
| *An. jamesi* | 0.38 | 0.54 | 38.3 | – | – | – | 0.29 | 0.47 | 23.08 |
| *An. karwari* | – | 0.35 | 25.53 | – | – | – | – | 0.31 | 15.38 |
| *An. kochi* | 5.87 | 3.39 | 87.23 | 3.64 | 4.50 | 70.97 | 5.35 | 3.52 | 80.77 |
| *An. maculatus* | 2.74 | 0.98 | 51.06 | 2.85 | 2.96 | 67.74 | 2.77 | 1.22 | 57.69 |
| *An. minimus* | 4.65 | 2.93 | 74.47 | 4.33 | 3.31 | 83.87 | 4.58 | 2.97 | 78.21 |
| *An. philippinensis/nivipes* | 10.34 | 9.89 | 100 | 12.3 | 14.15 | 87.10 | 10.8 | 10.39 | 94.87 |
| *An. subpictus* | – | 0.06 | 12.77 | – | – | – | – | 0.05 | 7.69 |
| *An. tessellatus* | – | 0.08 | 12.77 | 0.91 | 0.56 | 32.26 | 0.21 | 0.13 | 20.51 |
| *An. varuna* | – | 0.05 | 25.53 | 0.46 | 0.28 | 19.35 | 0.11 | 0.07 | 23.08 |
| *An. vagus* | 6.07 | 8.71 | 100 | 7.97 | 8.23 | 77.42 | 6.52 | 8.65 | 91.03 |
| *Cx. epidesmus* | – | – | – | 0.68 | 0.42 | 12.90 | 0.16 | 0.05 | 5.13 |
| *Cx. quinquefasciatus* | 5.55 | 8.62 | 100 | 13.44 | 9.71 | 100 | 7.40 | 8.75 | 100 |
| *Cx. tritaeniorhynchus* | 0.10 | 0.67 | 61.7 | 0.34 | 0.56 | 38.71 | 0.16 | 0.66 | 52.56 |
| *Cx. malayi* | 7.98 | 7.11 | 74.47 | 4.56 | 5.07 | 61.29 | 7.18 | 6.87 | 69.23 |
| *Cx. gelidus* | 1.11 | 1.97 | 51.06 | 2.28 | 2.39 | 67.74 | 1.38 | 2.02 | 57.69 |
| *Cx. vishnui* | 19.19 | 23.64 | 87.23 | 30.87 | 32.51 | 100 | 21.92 | 24.69 | 92.31 |
| *Ar. subalbatus* | 6.39 | 14.72 | 87.23 | 6.38 | 6.97 | 87.10 | 6.38 | 13.8 | 87.18 |
| *Aedes albopictus* | 0.14 | 0.27 | 38.3 | 0.91 | 0.77 | 51.61 | 0.32 | 0.33 | 56.41 |
| *Ma. annulifera* | – | 0.08 | 12.77 | – | – | – | – | 0.07 | 7.69 |
| *Ma. indiana* | 0.52 | 0.37 | 61.7 | 1.59 | 0.99 | 16.13 | 0.77 | 0.44 | 43.59 |
| *Ma. uniformis* | 0.69 | 1.20 | 61.7 | 2.62 | 3.10 | 58.06 | 1.14 | 1.42 | 60.26 |
| *Coquillettidia crassipes* | – | – | – | 0.57 | 0.35 | 9.68 | 0.13 | 0.04 | 3.85 |

TABLE 2. Temporal variability in mosquito species composition in South and North districts of Tripura State (2006-08)

| Mosquito species | South Tripura | | North Tripura | |
|---|---|---|---|---|
| | Pre-monsoon | Monsoon | Pre-monsoon | Monsoon |
| *An. aconitus* | + | − | + | + |
| *An. annularis* | + | − | − | − |
| *An. barbirostris* | + | + + + | + | + |
| *An. crawfordi* | ++ | ++ | + | − |
| *An. culicifacies* | + | − | − | − |
| *An. dirus* | − | + | − | + |
| *An. fluviatilis* | − | + | − | − |
| *An. jamesi* | + | + | − | − |
| *An. karwari* | + | + | − | − |
| *An. kochi* | + | ++ | − | + |
| *An. maculatus* | − | + | + | + |
| *An. minimus* | + | + | + | + |
| *An. philippinensis/nivipes* | + | ++ | − | ++ |
| *An. subpictus* | + | − | − | − |
| *An. tessellatus* | + | − | + | + |
| *An. varuna* | + | + | − | + |
| *An. vagus* | + | + + + | ++ | ++ |
| *Cx. epidesmus* | − | − | − | + |
| *Cx. quinquefasciatus* | + | + + + | ++ | ++ |
| *Cx. tritaeniorhynchus* | + | + | − | + |
| *Cx. malayi* | + | + + + | ++ | + |
| *Cx. gelidus* | − | + | − | + |
| *Cx. vishnui* | + + + | + + + | + + + | + + + |
| *Ar. subalbatus* | + | + + + | + | ++ |
| *Aedes albopictus* | + | + | + | + |
| *Ma. annulifera* | − | + | − | − |
| *Ma. indiana* | + | + | + | − |
| *Ma. uniformis* | + | + | + | + |
| *Coquillettidia crassipes* | − | − | + | − |
| Total number | 22 | 22 | 15 | 19 |

+ sparse; ++ moderate; + + + wide.

to the genus *Anopheles*. In the North, 22 species were recorded of which 11 were anophelines. The number of species of *Culex* recorded in the South and North were five and six, respectively. Mosquitoes of the genera *Armigeres*, *Mansonia* and *Aedes* were also obtained from both the districts. *Coquillettidia crassipes* was recorded in low density from the North.

The seasonal prevalence of the mosquitoes is shown in Table 2. Twenty two species were observed in the South during pre-monsoon season while only 15 were observed in the North. The number of species recorded during the monsoon season was 22 in the South and 19 in the North.

TABLE 3. Changes in species composition of *Anopheles* mosquitoes in Tripura over the years

| Species | Percent adult collection in light traps | | |
|---|---|---|---|
| | 1991* | 1998** | 2006–08 |
| *Anopheles aconitus* | 4.24 | 0.00 | 0.51 |
| *An. annularis* | 0.00 | 0.00 | 0.04 |
| *An. barbirostris* | 5.26 | 25.20 | 14.07 |
| *An. culicifacies* | 0.06 | 0.00 | 0.41 |
| *An. crawfordi* | 10.01 | 0.00 | 16.70 |
| *An. fluviatilis* | 0.00 | 0.00 | 0.08 |
| *An. hyrcanus* | 0.00 | 0.59 | 0.00 |
| *An. jamesi* | 6.94 | 0.00 | 1.16 |
| *An. jeyporiensis* | 0.00 | 3.52 | 0.00 |
| *An. karwari* | 25.78 | 10.16 | 0.75 |
| *An. kochi* | 5.16 | 13.09 | 8.62 |
| *An. dirus* | 0.13 | 0.00 | 0.14 |
| *An. maculatus* | 0.01 | 0.00 | 2.98 |
| *An. minimus* | 5.18 | 10.16 | 7.28 |
| *An. nigerrimus* | 0.14 | 0.00 | 0.00 |
| *An. majidi* | 0.10 | 0.00 | 0.00 |
| *An. pallidus* | 0.85 | 0.00 | 0.00 |
| *An. philippinensis/nivipes* | 29.76 | 29.49 | 25.45 |
| *An. pseudojamesi* | 0.00 | 0.59 | 0.00 |
| *An. subpictus* | 0.25 | 0.00 | 0.12 |
| *An. tessellatus* | 0.14 | 0.00 | 0.33 |
| *An. vagus* | 5.99 | 3.13 | 21.18 |
| *An. varuna* | 0.00 | 4.10 | 0.18 |

*Das *et al.*(1991), **Prakash *et al.*1998.

*An. minimus* was recorded with a percent distribution of 78.21 in the region and formed 4.58% of the indoor collections and 2.97% of the light trap collections. The North district was found to have a higher population of the species than the South. *An. minimus* is recognised as a principal vector of malaria in the northeastern India (Baruah *et al.*, 2004). *An. dirus* was sparsely distributed (19.23%) and formed 0.19% of the indoor collection. This species is a major vector of malaria in the northeastern India but is not incriminated in Tripura (Malhotra, 2000). It was also reported from Tamil Nadu, Andaman Islands, Karnataka and Kerala (Nagpal and Kalra, 1997). *An. dirus* complex comprise at least seven species of which species D is found exclusively in Myanmar, Bangladesh and in the northeastern states of India (Subbarao, 1998). This species is considered responsible for the spread of mutant drug resistant strain of *P. falciparum* malaria in the region. Modelling studies indicated that 3–10% areas in Tripura are favourable to *An. dirus* (Srivastava *et al.*, 2001).

*An. philippinensis/nivipes* was widely distributed in the region (94.87%) and formed 10.8% and 10.39%, respectively of the indoor resting and light trap collections. This complex is likely to be involved in malaria transmission in the northeastern states

(Prakash *et al.*, 2005) and is considered to be a vector of local importance in India (Dash *et al.*, 2007).

*An. fluviatilis* was found to be sparsely distributed in the South while it was not recorded from the North. It is a primary vector of malaria in the foothill regions of India (Sharma, 1998) and is considered as a vector in Pakistan and Nepal (Rao, 1984). *An. culicifacies* was recorded in small numbers from the South in the pre-monsoon season. This species is widely distributed throughout the country and contributes to about 60–65% of all malaria cases (Sharma, 1998). It is a major vector in Iran, Afghanistan, Pakistan and Sri Lanka (Subbarao, 1998).

*An. subpictus* was recorded from some sites in the South. It is a vector of human filariasis in India (Raghavan, 1969) and the main vector of malaria in Sri Lanka (Panicker *et al.*, 1981). It is considered as a non-vector species in India though infected specimens with malarial parasite have been reported (Kirti and Kaur, 2004). It is considered to be a secondary vector in the zoonotic transmission of Japanese encephalitis (JE) (Samuel, 2000). *An. barbirostris* formed 12.02% of the indoor collections and 5.75% of the light trap collections, with a percent distribution of 66.67 and was found in high density during monsoon season in the South. It is a minor vector of filariasis and a suspected vector of JE in India (Samuel, 2000; Chakravarty *et al.*, 1975).

*An. aconitus* was sparsely distributed in the South and moderately, in the North. It is a secondary vector of malaria in India. The potential of this species as a malaria vector in Tripura needs to be investigated considering its incrimination in neighbouring Bangladesh (Maheswary *et al.*, 1992; Malhotra, 2000). *An. tessellatus* is a minor vector in Indonesia and Maldives and is suspected to be a vector in India (Malhotra, 2000; Nagpal and Sharma, 1995). This species was recorded in low numbers from both districts (20.51%). *An. maculatus* is a suspected vector of malaria in the northeastern India (Malhotra, 2000) and was moderately distributed (57.69%). It was recorded in both the seasons in the North but only during the monsoon season from the South, and formed 2.77% of indoor resting collections and 1.22% of the light trap catches. *An. annularis* is a secondary vector of malaria (Nagpal and Sharma, 1995), and was recorded in low numbers in the South. *An. varuna*, which is a vector of local importance in India (Dash *et al.*, 2007) was sparsely distributed. *An. vagus* was widely distributed (91.03%) and was collected from all the sites in the South. It formed 6.52% of the indoor resting collections and 8.65% of the light trap collections. *An. kochi* and *An. crawfordi*, which are considered to be non-vectors were widely distributed in the region (80.77% and 66.67%) while *An. jamesi* and *An. karwari* were observed only in collections from the South.

*Culex quinquefasciatus* was the most widely distributed species in the two districts (100%) and was abundant in both pre-monsoon and monsoon seasons. It was collected from all the sites and formed 7.4% of the indoor resting collections and 8.75% of the light trap collections. It is the major vector of bancroftian filariasis in urban areas (Samuel *et al.*, 2004). It is a strongly anthropophilic species which has been shown to be able to transmit JE virus under laboratory conditions (Banerjee *et al.*, 1977).

*Cx. vishnui* group of mosquitoes including *Cx. tritaeniorhynchus* and *Cx. vishnui* play an important role in the transmission of JE in India (Samuel, 2000). The percent distributions for these two species were 52.56 and 92.31, respectively. *Cx. vishnui* formed 21.92% of indoor collections and 24.69% of light trap collections, indicating that *Cx. vishnui* was the most abundant and widely distributed species in the region. *Cx. gelidus* is one of the vectors of JE in India (Tilak *et al.*, 2008), which was recorded from both the districts with a distribution of 57.69%. This species formed 1.38% of the indoor resting collections and 2.02% of the light trap collections. *Cx. malayi* was widely distributed in the two districts (69.23%). *Cx. epidesmus* and *Coquillettidia crassipes* were collected in small numbers from the North during monsoon and pre-monsoon season, respectively.

Among the *Mansonia* species, *Ma. uniformis* was the most widely distributed (60.26%) followed by *Ma. indiana* (43.59%). *Ma. annulifera* was observed in light trap collections from some areas in the South. *Ma. annulifera* is the principal vector of Malayan filariasis in rural India, while *Ma. uniformis* is the secondary vector (Agrawal and Sashindran, 2006). The *Mansonia* species are suspected vectors of JE in India (Samuel, 2000; Chakravarty *et al.*, 1981). *Armigeres subalbatus* was abundant in both the districts with a percent distribution of 87.18. It formed 6.38% of indoor resting collections and 13.8% of light trap collections. It is an efficient vector of JE and filariasis and also creates nuisance due to its diurnal man biting nature (Malhotra, 2000). *Aedes albopictus*, a secondary vector of dengue and dengue hemorrhage fever (Shetty *et al.*, 2007) was collected from 44 sites in the region.

The species composition of anopheline mosquitoes in the State has apparently changed during the last few decades (Table 3). The mosquito surveys in Tripura by Misra and Dhar (1955) indicated that there were 10 species of *Anopheles* in the region among which *An. vagus* (80%) and *An. minimus* (13%) were the predominant ones. Das *et al.* (1991) reported the presence of 17 species of anophelines of which *An. philippinensis* (29.76%) and *An. karwari* (25.78%) were the most abundant. Prakash *et al.* (1998) observed the presence of 10 species of *Anopheles* in light trap collections, *An. philippinensis/nivipes* and *An. barbirostris* forming 29.49 and 25.20% of the collection, respectively. In the present study, 17 species of *Anopheles* were recorded, of which *An. philippinensis/nivipes* (25.45%), *An. vagus* (21.18%), *An. crawfordi* (16.70%) and *An. barbirostris* (14.07%) were the predominant ones.

The studies on biting activity indicated that *An. philippinensis/nivipes* had the highest mean human landing rate of 2.8 per man per hour (Fig. 1) followed by *An. crawfordi* (2.1) and *An. minimus* (2). The landing rate for other species were *Cx. quinquefasciatus*, 1.6; *Cx. vishnui*, 1.4; *An. barbirostris*, 1.8; *An. karwari*, 1.2; *Cx. malayi*, 1.1; and *Cx. gelidus*, 0.6. The highest human landing rate was recorded during 0200–0300 h for *Anopheles* (21.6) and during 2000–2100 h for *Culex* (10.8). The mean landing rate of *Anopheles* observed across all sites during the study period ($9.9 \pm 2.0$) was significantly higher ($t = 2.38, df = 22; p < 0.05$) than that of *Culex* ($4.6 \pm 0.98$).

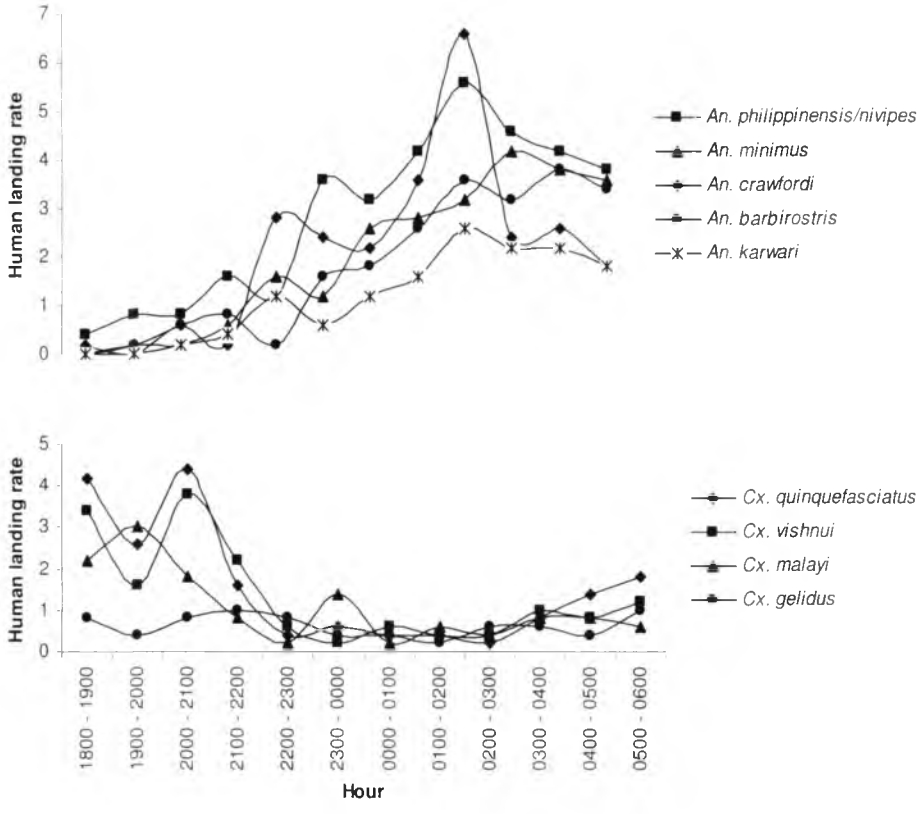


FIGURE 1. Hourly indoor human landing frequency of *Anopheles* and *Culex* mosquitoes in Tripura (2006–08).

The present studies were undertaken in order to understand the diversity and distribution of mosquito vectors in the State of Tripura, in the northeastern region of India. Tripura has remained largely unexplored with regard to the entomological studies on vectors of human diseases. Extensive studies on the distribution, seasonal prevalence and biting activity of mosquito species have been conducted for the first time in the State. The changes in species composition of mosquitoes over the decades have been elucidated. Information on the distribution of vectors of infectious diseases can help us to identify and target the areas with potential for disease outbreak.

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# Dung beetle (Coleoptera: Scarabaeidae and Aphodiidae) diversity and resource utilisation within a protected area in Swaziland

**Cebisile N. Magagula**

*Department of Biological Sciences, University of Swaziland, P/Bag 4, Kwaluseni, Swaziland*
*Email: Cebisile@uniswacc.uniswa.sz*

ABSTRACT: The investigation was carried out at Mlawula Nature Reserve, a protected area close to sugarcane plantations, over a six month period from September to February. Population and species diversity survey yielded 11,834 dung beetles, representing 64 species (51 Scarabaeidae and 13 Aphodiidae). December had the highest diversity (53 species) while January had the highest population densities (4179). Genus *Onthophagus* (13 species; 4722 beetles) was the most dominant (39.90% of total). *O. interstitialis* was the dominant species (19.02% of total), followed by *Sisyphus seminulum* (18.06% of total). Genus *Aphodius* also had 13 species but fewer individuals were collected. All guilds were represented, with tunnellers being the most dominant. The beetles' utilisation of dung as it aged was investigated at bimonthly intervals, with three dung treatments over a 48 h period. A total of 6384 beetles (32 species) were collected. Significant differences between treatments were observed ($p = 0.001$), e.g. *O. interstitialis*, *Drepanocerus kirbyi* and *Sarophorus costatus* dominated in the three treatments, illustrating temporal differentiation in resource utilisation. The study emphasised the value of natural habitats, as found in nature reserves, for the maintenance of landscape heterogeneity and to promote overall diversity compared to the surrounding agricultural landscape.


KEYWORDS: dung beetles, Scarabaeidae, Aphodiidae, insect conservation, diversity

## INTRODUCTION

Invertebrates, particularly insects, have an irreplaceable contribution to ecosystem function and processes. They have been increasingly used in evaluation of conservation efforts as well as biodiversity monitoring, assessment and conservation (Dangerfield *et al.*, 2003; New, 2007). Their capacity for local habitat and resource specialisation makes them useful as indicators of ecosystem health than larger animals.

This implies that there are a range of scales at which habitat heterogeneity may affect assemblage patterns of invertebrates than the traditionally studied vertebrate and plant taxa. In particular, beetles make up the most organismal variability in all habitats and are good candidates for quantitative biodiversity evaluation. Their diverse ecological roles and strategies enable analysis of factors affecting biodiversity (Duelli *et al.*, 1999; Romero-Akaraz and Avilla, 2000; Davis *et al.*, 2001; Melnychuk *et al.*, 2003).

The key to the protection of any species, including insects, is the protection of its habitat (Van Rensburg *et al.*, 1999). Increasing human impact due to landscape modifications and transformation, e.g. agricultural activity, has lead to the reduction in intact habitats, with detrimental effects on biodiversity. Intact habitats, such as conservation areas, are thus important for preservation of natural habitats and make significant contribution towards conservation strategies, especially for the invertebrate taxa (Koch *et al.*, 2000; Samways, 2005). Natural habitats embedded within agricultural areas also act as home to insect diversity. However, such areas need to be comprehensive enough to cover all taxa, so consideration of their size and habitat quality should be included.

Swaziland has a number of protected areas proclaimed to promote biodiversity conservation. While the higher plant and animal taxa in Mlawula Nature Reserve, a conservation area in the lowveld of Swaziland, have been well documented (Monadjem, 1998; Monadjem *et al.*, 2003; Loffler and Loffler, 2005), no survey on dung beetle taxa has been done. Hence, this study was carried out to ascertain the population densities and species diversity of dung beetles (Coleoptera: Scarabaeidae and Aphodiidae) within the reserve. The dung beetle community is often abundant, with several individuals attracted to the same, ephemeral resource. Utilisation of the same resource by the dung beetles will thus impact on population dynamics and interactions between and within species (Hanski and Cambefort, 1991a,b). This study also sought to investigate changes in the dung beetle community as the dung aged.

## MATERIALS AND METHODS

### Study area

The study was carried out at Mlawula Nature Reserve located in north-eastern Swaziland, (26°09′E and 26°20′E, 31°56′S and 32°06′S) covering an area of approximately 16,500 ha. The reserve is situated within a transitional zone between two geographic regions, i.e. the lowveld plains with dry thorny savanna vegetation on the west and the Lubombo mountain range with broadleaved woodlands, ironwood forests and moist, coastal thickets on the east. The reserve consists of three distinctive ecological zones, namely the Ndzindza plateau, Siphiso valley and rhyolitic ridges on the western boundary. The reserve is close to other protected areas (Mbuluzi and Simunye Nature Reserves, Hlane National Park), with the surrounding landscape dominated by sugarcane plantations. Average annual rainfall is between 500–600 mm. Mid-winter temperatures range between 10–20 °C and mid-summer temperatures, between 22–36 °C (Boycott *et al.*, 2007; SNTC, 2008).

## Sampling

The survey was carried out in the Siphiso valley, where three sampling sites were randomly selected. Stratified random sampling was used to ensure that a diversity of vegetation covers was represented. The first site was characterised by thorny bushes, 1.5–2 m high and clumpy grass that grew up to 1.5 m. Soils were silt and homogenous light brown in colour. Site two was open, mixed woodland characterised by common woody species like *Acacia nigrescens*. The under storey was dominated by *Parthenium hysterophoris*, an annual invasive species, which grew up to 1 m height. The soils were sandy-loam and homogenous light brown. Site three was a moist broad-leaved savanna characterised by broadleaved, often deciduous species and sedges. The soils were moist, clay loam and dark grey in colour.

Sampling for population and species diversity was carried out once a month on two consecutive days for a period of six months from September 2003 to February 2004. Baited pitfall traps were used to collect the beetles, with fresh cow dung collected from grazing areas outside the reserve. Each trap consisted of an 8 × 10 cm polyvinyl pipe, forming the outer cover and an inner plastic cup. The pipes were dug into the ground so that the top was flush with the soil surface and remained in the ground until the end of the survey. The plastic cups were then 3/4-filled with fresh cow dung and inserted into the pipes so that only the cups were changed during servicing of the traps. At each sampling site, pitfall traps were set out in three transects, 10 m apart. Each transect was 18 m long with 4 traps six metres apart so that a total of 12 traps were at each site and 36 in total. The traps were baited twice a day, i.e. morning and late afternoon, with samples collected with each change and combined for each day.

An additional experiment at the same site was carried out to assess changes in resident species in the dung variously exposed over 48 h. This was carried out at sites one and three during October, December and February. At each site two grids, each measuring 3 m × 3 m and consisting of nine pitfall traps, were prepared. There were three treatments, randomly arranged in each grid. Treatment one was fresh dung exposed for 24 h, treatment two was dung exposed for 48 h and treatment three consisted of dung covered with a fine net to exclude beetles and exposed for 24 h, followed by a further 24 h with the netting removed. Each treatment was replicated three times in each grid, so that each site had 18 traps and a total of 36 traps. An extra pitfall trap was dug at each site and was used to observe changes in moisture content over the 48 h period. Moisture content of the dung was assessed by weighing 10 g of dung, placing it uncovered in a petri-dish in an oven for 48 h at 85° C and reweighing after 48 h.

All sampling cups were emptied into individually labelled plastic. Dung beetles were then separated by pouring water into the bags and the floating beetles removed. The remaining contents were emptied over a sieve tower arranged in decreasing perforation size to collect varying sizes of dung beetles. Several washes were done until all beetles were removed from the dung. Initially, beetles collected were separated into different species, then identified using reference collections in the Swaziland National Museum and the Department of Biological Sciences (University

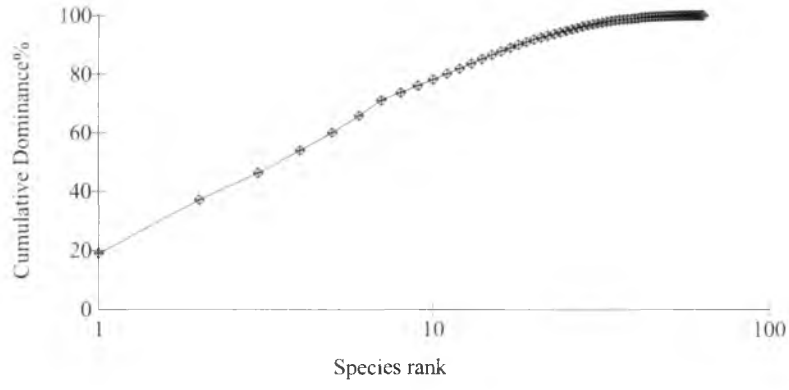


FIGURE 1. Species cumulative curve for dung beetles collected from Mlawula Nature Reserve.

of Swaziland). Subsequently, beetles were identified, counted and released in the field and only new specimens kept for identification.

## RESULTS

### Population and Diversity survey

A total of 11,834 dung beetles, representing 64 species (51 Scarabaeidae and 13 Aphodiidae), were collected from the reserve during the sampling period and of these, only 4 species were unidentified, from the reference collections (Table 1). The cumulative species curve (Fig. 1) illustrated a gradual increase in number of species with sampling effort and eventually levelled off, indicating that further sampling would not have significantly increased species numbers. There were no significant differences in number of individuals and species collected between the three sites ($p > 0.05$) and data were therefore pooled from all sites. Monthly analyses showed significant differences in the number of individuals collected (Global $R = 0.386$, $p = 0.001$) (Fig. 2). October had the lowest species diversity and number of dung beetles collected. Numbers and species diversity subsequently increased progressively with season and dung beetle abundance was highest in January, which was two months after the highest rainfall was recorded during the sampling period. Species diversity was highest (53 spp.) in December. This period coincided with seasonal maximisation of dung beetle species richness in this region (Davis, 1996; Stronkhorst and Stronkhorst, 1998). Both species diversity and population densities had sharply declined when sampling stopped in February.

The genus *Onthophagus* was the most dominant in the sample, with 4722 individuals of 13 species, making up 39.9% of the total, followed by *Sisyphus* with 2858 individuals making up 24.15% (Table 2). Unlike *Onthophagus*, this genus was represented by only two species. It can be seen from the species dominance plot (Fig. 3) that one species, *Onthophagus interstitialis*, made up 19.02% of the total sample (and

TABLE 1. Dung beetle populations and species collected from three sites over two sampling days from Mlawula Nature Reserve

| Species & Guild | Site 1 | Site 2 | Site 3 | Total | % of total |
|---|---|---|---|---|---|
| *Anachalcos convexus* (R) | 30 | 50 | 45 | 125 | 1.06 |
| *Aphodius colobopterus maculicollis* (D) | 1 | 0 | 0 | 1 | 0.01 |
| *Aphodius* sp. (D) | 0 | 9 | 7 | 16 | 0.14 |
| *Aphodius* sp.1 (D) | 2 | 8 | 5 | 15 | 0.13 |
| *Aphodius* sp.10 (D) | 1 | 3 | 5 | 9 | 0.08 |
| *Aphodius* sp.11 (D) | 0 | 4 | 3 | 7 | 0.06 |
| *Aphodius* sp.12 (D) | 0 | 0 | 2 | 2 | 0.02 |
| *Aphodius* sp.13 (D) | 18 | 58 | 0 | 76 | 0.64 |
| *Aphodius* sp.4 (D) | 1 | 6 | 0 | 7 | 0.06 |
| *Aphodius* sp.5 (D) | 1 | 9 | 3 | 13 | 0.11 |
| *Aphodius* sp.6 (D) | 0 | 4 | 2 | 6 | 0.05 |
| *Aphodius* sp.7 (D) | 1 | 17 | 9 | 27 | 0.23 |
| *Aphodius* sp.8 (D) | 20 | 6 | 23 | 49 | 0.41 |
| *Aphodius* sp.9 (D) | 4 | 5 | 24 | 33 | 0.28 |
| *Caccobius* sp. (T) | 222 | 385 | 307 | 914 | 7.72 |
| *Copris amyntor* (T) | 26 | 62 | 50 | 138 | 1.17 |
| *Copris elphenor* (T) | 3 | 2 | 5 | 10 | 0.08 |
| *Copris evenidus* (T) | 1 | 2 | 2 | 5 | 0.04 |
| *Copris hidius* (T) | 48 | 1 | 26 | 75 | 0.63 |
| *Copris* sp.1 (T) | 36 | 10 | 13 | 59 | 0.50 |
| *Cyptochirus s.p* (T) | 21 | 7 | 6 | 34 | 0.29 |
| *Digitonthophagus gazella* (T) | 2 | 13 | 0 | 15 | 0.13 |
| *Drepanocerus kirbyi* (T) | 125 | 42 | 40 | 207 | 1.75 |
| *Drepanocerus* sp. (T) | 53 | 22 | 29 | 104 | 0.88 |
| *Euoniticellus intermedius* (T) | 5 | 6 | 0 | 11 | 0.09 |
| *Euoniticellus* sp.1 (T) | 36 | 17 | 10 | 63 | 0.53 |
| *Euoniticellus* sp.2 (T) | 0 | 2 | 0 | 2 | 0.02 |
| *Garreta nitens* (R) | 60 | 73 | 148 | 281 | 2.37 |
| *Hyalonthophagus aleyonides* (D) | 14 | 17 | 27 | 58 | 0.49 |
| *Kepher nigroaeneus* (R) | 2 | 9 | 6 | 17 | 0.14 |
| *Kepher* sp.1 (R) | 39 | 67 | 60 | 166 | 1.40 |
| *Kepher* sp.2 (R) | 0 | 1 | 6 | 7 | 0.06 |
| *Liatongus militaris* (T) | 2 | 5 | 0 | 7 | 0.06 |
| *Milichus apicalis* (T) | 64 | 84 | 152 | 300 | 2.54 |
| *Neosisiphus* sp. (R) | 17 | 7 | 3 | 27 | 0.23 |
| *Neosisyphus fortuitus* (R) | 91 | 78 | 68 | 237 | 2.00 |
| *Neosisyphus ruber* (R) | 35 | 0 | 4 | 39 | 0.33 |
| *Oniticellus planatus* (D) | 2 | 0 | 1 | 3 | 0.03 |
| *Onitis alexis* (T) | 1 | 0 | 5 | 6 | 0.05 |
| *Onitis fulgidus* (T) | 171 | 250 | 263 | 684 | 5.78 |
| *Onitis picticollis* (T) | 0 | 2 | 0 | 2 | 0.02 |
| *Onitis* sp.1 (T) | 0 | 0 | 2 | 2 | 0.02 |

TABLE 1. (Contd...)

| Species & Guild | Site 1 | Site 2 | Site 3 | Total | % of total |
|---|---|---|---|---|---|
| *Onitis viridulus* (T) | 1 | 1 | 3 | 5 | 0.04 |
| *Onthophagus aeruginosus* (T) | 57 | 46 | 85 | 188 | 1.59 |
| *Onthophagus corniculiger* (T) | 1 | 0 | 0 | 1 | 0.01 |
| *Onthophagus fimetrarius* (T) | 35 | 27 | 6 | 68 | 0.57 |
| *Onthophagus interstitialis* (T) | 232 | 1206 | 813 | 2251 | 19.02 |
| *Onthophagus lamelliger* (T) | 199 | 412 | 477 | 1088 | 9.19 |
| *Onthophagus nr pullus* (T) | 15 | 22 | 3 | 40 | 0.34 |
| *Onthophagus nr sugillatus* (T) | 66 | 42 | 37 | 145 | 1.23 |
| *Onthophagus obtusicornis* (T) | 1 | 2 | 1 | 4 | 0.03 |
| *Onthophagus pallidipennis* (T) | 0 | 1 | 0 | 1 | 0.01 |
| *Onthophagus rasipennis* (T) | 94 | 41 | 68 | 203 | 1.72 |
| *Onthophagus* sp.1 (T) | 185 | 113 | 324 | 622 | 5.26 |
| *Onthophagus* sp.2 (T) | 25 | 34 | 51 | 110 | 0.93 |
| *Onthophagus* sp.3 (T) | 1 | 0 | 0 | 1 | 0.01 |
| *Proagodenus tersidorsis* (T) | 9 | 10 | 40 | 59 | 0.50 |
| *Sarophorus costatus* (T) | 109 | 107 | 34 | 250 | 2.11 |
| *Sisyphus gazanus* (R) | 212 | 333 | 176 | 721 | 6.09 |
| *Sisyphus seminulum* (R) | 293 | 713 | 1131 | 2137 | 18.06 |
| *Unid* sp.3 (U) | 0 | 5 | 2 | 7 | 0.06 |
| *Unid* sp.1 (U) | 36 | 16 | 20 | 72 | 0.61 |
| *Unid* sp.4 (U) | 0 | 1 | 0 | 1 | 0.01 |
| *Unid* sp.5 (U) | 1 | 0 | 0 | 1 | 0.01 |
| Total beetles | 2727 | 4475 | 4632 | 11834 | 100 |
| Species | 53 | 55 | 51 | 64 | 64 |

Guilds abbreviated as D, Dweller; R, Roller; T, Tunneller; U, Unknown.

47.67% of individuals from the genus), followed by *Sisyphus seminulum* which made up 18.06% of the total (and 74.77% of individuals from the genus). Like *Sisyphus*, *Caccobius*, *Onitis*, etc., some genera were represented by only one or two species but still contributed significantly to the overall numbers (Table 2). Genus *Aphodius* had an equal number of species collected as *Onthophagus* but had far fewer beetles collected.

Based on their utilisation of the dung, beetles are divided into three guilds, i.e. tunnellers, rollers and dwellers. Species collected represented all three guilds, i.e. rollers (e.g. *S. seminulum, A. convexus, and S. gazanus*), tunnellers (e.g. *Onthophagus* species, *Drepanocerus* species, and *Onitis* species) and dwellers ( *Aphodius* species, *H. aleyonides*). Tunnellers were the most dominant group in terms of species diversity and abundance. Although rollers were next in abundance, they had fewer species than dwellers due to the dwellers' dominance by *Aphodius* species. Pairwise tests showed that the number of rollers was significantly different from dwellers ($R = 0.537$, $p = 0.001$) while no significant differences were observed between rollers and tunnellers ($R = 0.045$, $p = 0.225$), nor between tunnellers and dwellers ($R = -0.092$, $p = 0.96$).

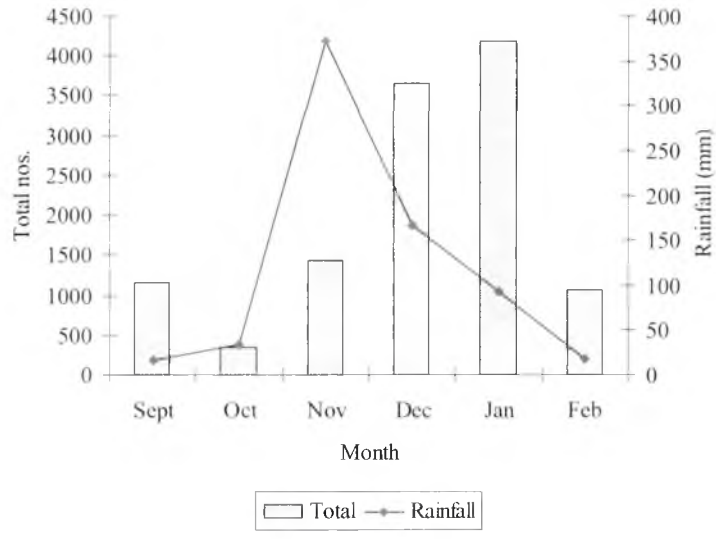


FIGURE 2. Dung beetle abundance and amount of rainfall per month during the sampling period.

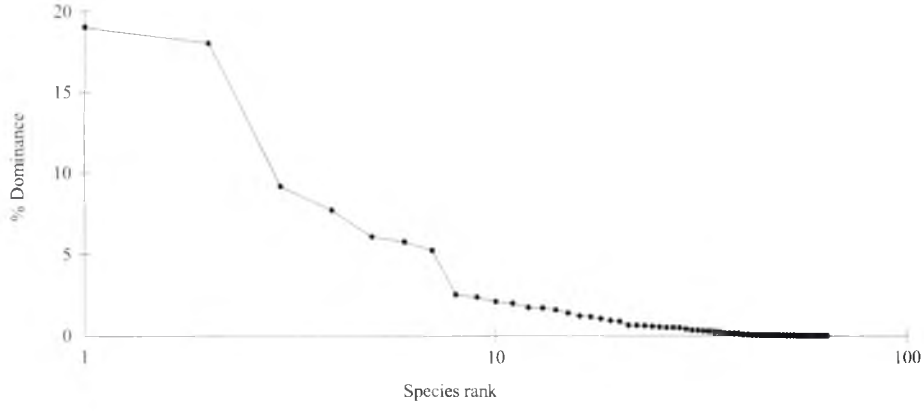


FIGURE 3. Species dominance curve for the dung beetles collected during the population and diversity survey.

## Effect of dung age on resident species

In the experiment to study the effect of dung age on its occupation by beetles, 6384 beetles, representing 32 species (27 Scarabaeidae and 5 Aphodiidae), were collected and only two of these were unidentified. As in the population and diversity survey, *Onthophagus interstitialis* was the most dominant species, making 27.26% of the beetles collected. There were no significant differences between sites and replicates

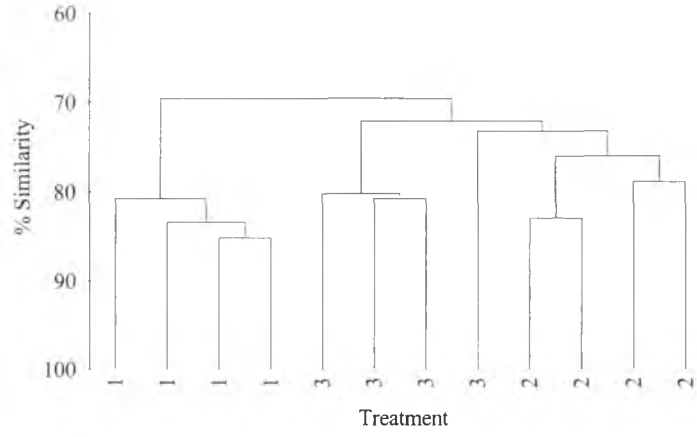


FIGURE 4. Cluster analysis of dung treatments, where treatment 1 is fresh dung exposed for 24 h; treatment 2 is fresh dung exposed for 48 h; treatment 3 is beetles excluded from exposed dung for 24 h and then exposed for 24 h.

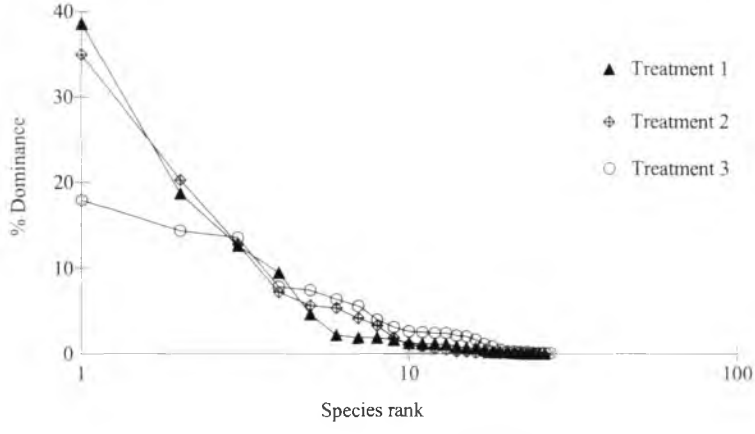


FIGURE 5. Species rank abundance curve for all treatments to show species dominance in each treatment, where treatment 1 is fresh dung exposed for 24 h; treatment 2 is fresh dung exposed for 48 h; treatment 3 is beetles excluded from exposed dung for 24 h and then exposed for a further 24 h.

and therefore the data were combined. Significantly higher densities were collected from treatment 1, i.e. fresh dung (Global $R = 0.789$; $p = 0.001$). Cluster analysis indicated two main clusters, with treatment 1 separated from treatments 2 and 3 (Fig. 4). This treatment had 3154 (49.40%) beetles of 26 species. Pairwise tests revealed that all treatments were significantly different from each other ($P < 0.001$), with clusters based on the species collected from each treatment. This was also observed in the differences in dominant species between treatments (Fig. 5). Treatment

TABLE 2. The total number of species collected for each genus at Mlawula Nature Reserve

| Genus | No. of species | Total beetles | Percentage |
|---|---|---|---|
| *Onthophagus* | 13 | 4722 | 39.90 |
| *Sisyphus* | 2 | 2858 | 24.15 |
| *Caccobius* | 1 | 914 | 7.72 |
| *Onitis* | 5 | 699 | 5.91 |
| *Drepanocerus* | 2 | 311 | 2.63 |
| *Neosisyphus* | 3 | 303 | 2.56 |
| *Milichus* | 1 | 300 | 2.54 |
| *Copris* | 5 | 287 | 2.44 |
| *Garreta* | 1 | 281 | 2.37 |
| *Aphodius* | 13 | 261 | 2.21 |
| *Sarophorus* | 1 | 250 | 2.11 |
| *Kepher* | 3 | 190 | 1.61 |
| *Anachalcos* | 1 | 125 | 1.06 |
| *Unld* sp | 4 | 81 | 0.68 |
| *Euoniticellus* | 3 | 76 | 0.64 |
| *Proagoderus* | 1 | 59 | 0.50 |
| *Hyalonthophagus* | 1 | 58 | 0.50 |
| *Cyptochirus* | 1 | 34 | 0.29 |
| *Digitonthophagus* | 1 | 15 | 0.13 |
| *Liatongus* | 1 | 7 | 0.06 |
| *Oniticellus* | 1 | 3 | 0.03 |
| Total | 64 | 11 834 | 100 |

1 was dominated by *O. interstitialis*, which made up 38.62% in this treatment. In treatments 2 and 3 this beetle decreased to 20.24% and 7.47%, respectively, indicating a reduction of its numbers as the dung aged. Treatment 2 was dominated by *Drepanocerus kirbyi*, which made up 34.97% in the treatment and treatment 3 was dominated by *Sarophorus costatus*, at 17.94%. Treatment 3 had the lowest number of beetles, but the beetle diversity was not significantly different from the other treatments (Table 3). There was a reduction in the number of beetles as the dung aged. Moisture content decreased over the 48 h but there was no significant correlation between population densities and moisture content ($r = -0.2455$; $p = 0.4420$).

In all treatments, the dominant species was a tunneller. Guild analyses indicated that there were no significant differences in beetles relative to their utilisation of the dung ($P > 0.05$). However, some species had preferences for the dung at different ages as shown by their absence from other treatments. For example, *Drepanocerus* sp. and *Aphodius* sp. 7 showed preference for fresh dung since they were collected only from treatment 1. Although collected from all treatments, some species had higher abundance in treatment 1 compared to the other treatments, e.g. *O. interstialis*, *O. lamelliger*, *Onitis fulgidus* and *Aphodius* sp. 9. *Neosisyphus ruber* and *Aphodius colobopterus maculicollis* were found only in treatment 3, while others like *Caccobius*

TABLE 3. Dung beetles collected from the different treatments of dung

| Species | T1 | T2 | T3 | Total | % of total |
|---|---|---|---|---|---|
| *Onthophagus interstitialis* | 1218 | 445 | 77 | 1740 | 27.26 |
| *Drepanocerus kirbyi* | 589 | 769 | 140 | 1498 | 23.46 |
| *Onthophagus lamelliger* | 399 | 92 | 81 | 572 | 8.96 |
| *Sisyphus gazanus* | 147 | 159 | 148 | 454 | 7.11 |
| *Onitis fulgidus* | 300 | 119 | 25 | 444 | 6.95 |
| *Sarophorus costatus* | 39 | 125 | 185 | 349 | 5.47 |
| *Milichus apicalis* | 7 | 282 | 41 | 330 | 5.17 |
| *Onthophagus rasipennis* | 51 | 72 | 58 | 181 | 2.84 |
| *Garreta nitens* | 70 | 41 | 32 | 143 | 2.24 |
| *Onthophagus pallidipennis* | 60 | 3 | 27 | 90 | 1.41 |
| *Onthophagus* sp. 1 | 37 | 24 | 17 | 78 | 1.22 |
| *Caccobius* sp. | 3 | 0 | 66 | 69 | 1.08 |
| *Aphodius* sp. 9 | 59 | 6 | 2 | 67 | 1.05 |
| *Anachalcos convexus* | 35 | 13 | 2 | 50 | 0.78 |
| *Aphodius* sp. 1 | 21 | 0 | 21 | 42 | 0.66 |
| *Kepher* sp. 1 | 14 | 18 | 9 | 41 | 0.64 |
| *Cyptochirus* sp. | 1 | 11 | 26 | 38 | 0.60 |
| *Aphodius* sp. 7 | 37 | 0 | 0 | 37 | 0.58 |
| *Proagoderus tersidorsis* | 22 | 2 | 12 | 36 | 0.56 |
| *Hyalonthophagus aleyonides* | 6 | 2 | 22 | 30 | 0.47 |
| *Drepanocerus* sp. | 27 | 0 | 0 | 27 | 0.43 |
| *Neosisyphus ruber* | 0 | 0 | 25 | 25 | 0.39 |
| *Unid* sp. 2 | 1 | 5 | 5 | 11 | 0.17 |
| *Aphodius* sp. 6 | 7 | 2 | 0 | 9 | 0.14 |
| *Onitis picticollis* | 2 | 3 | 0 | 5 | 0.08 |
| *Onthophagus nr pullus* | 1 | 3 | 1 | 5 | 0.08 |
| *Onitis viridulus* | 0 | 1 | 3 | 4 | 0.06 |
| *Aphodius colobopterus maculicollis* | 0 | 0 | 3 | 3 | 0.05 |
| *Liatongus militaris* | 0 | 1 | 1 | 2 | 0.03 |
| *Unid* sp. 6 | 0 | 1 | 1 | 2 | 0.03 |
| *Copris amyntor* | 1 | 0 | 0 | 1 | 0.02 |
| *Kepher nigroaeneus* | 0 | 0 | 1 | 1 | 0.02 |
| Total number of beetles | 3154 | 2199 | 1031 | 6384 | 100 |
| Total number of species | 26 | 24 | 27 | 32 | 32 |

T1, Fresh dung exposed for 24 h; T2, Fresh dung exposed for 48 h; T3, Beetles excluded from exposed dung for 24 h and then exposed for a further 24 h.

sp.1, *Cyptochirus* sp. and *Hyalonthophagus aleyonides* increased in numbers as the dung aged. No species showed preference for treatment 2, although notably higher numbers of *Milichus apicalis* were collected from this treatment than the other two (Table 3). Additionally, certain species had no preference for any treatment, e.g. *Sisyphus gazanus*, *O. rasipennis* and *Garreta nitens*.

## DISCUSSION

A total of 64 species (51 Scarabaeidae and 13 Aphodiidae) were collected from Mlawula Nature Reserve compared to 78 species (53 Scarabaeidae and 25 Aphodiidae) from Hlane Wildlife Sanctuary (Stronkhorst and Stronkhorst, 1998), located about 20 km away. While sampling duration at Mlawula Nature Reserve may not have yielded any critical differences in populations or species diversity, as confirmed by the cumulative curve obtained (Biaggini *et al.*, 2007), dung type and amount used in the two studies were different and could explain the differences observed. The analysis at Hlane was carried out in elephant dung pats.

The relatively high species diversity observed in Mlawula is in line with observations that summer rainfall regions of southern Africa have a comparatively high diversity in lowveld and highveld regions. Dung beetle association with rainfall was observed in this study, with dung beetle populations and diversity increasing to great abundance a few weeks after summer rains. Diversity in mammalian species also determines levels of resources available for the dung beetles (Hanski and Cambefort, 1991a). Mlawula reserve has a number of species of mammals, e.g. impala, baboons, wildebeest and livestock from neighbouring communities. With the exception of livestock, these mammalian species are absent in the agricultural landscape outside the reserve.

Differences in dung utilisation by dung beetles vary depending on their feeding preferences and reproductive strategies. In both experiments, the tunneller guild was the most dominant, having the largest number of beetles and species, which is in line with the findings that the tunneller and roller guilds are the most dominant guilds in this region. Although dung type was different, dominance by the tunneller guild was also observed in surveys carried out by Stronkhorst and Stronkhorst (1998) at Hlane Nature Reserve and Sabu *et al.* (2007) in Kerala State, India. The genera *Onthophagus* and *Aphodius*, which were the most speciose in this investigation, are also known to be the most species rich genera of dung beetles (Hanski and Cambefort, 1991a,b). This survey also corroborated dominance of the genus *Onthophagus* in this region, which was also observed in a coleopteran diversity survey carried out locally in an agricultural landscape at Tambuti (Magagula, 2006).

Due to the overlap in utilisation of the same resource, i.e. dung, conflict between and within species is expected. During this study, direct combat for possession of dung balls was observed between *A. convexus* individuals. The resource available was ephemeral and limited, yet more beetles converged than was needed for resource utilisation, leading to competition (Hanski and Cambefort, 1991a,b; Hunt *et al.*, 1999). In this study certain species like *Aphodius* sp. 7 and *Drepanocerus* species were collected only on fresh dung. These species colonise the dung quickly and need to have good competitive ability. On the other hand, species collected mainly from the 48 h old dung, e.g. *Neosisyphus ruber*, *Caccobius* sp. and *H. aleyonoides* were deduced to be slow colonisers and may be poor competitors (Hanski, 1991). Utilisation of the dung with temporal differentiation may be a competitive strategy utilised by the beetles to minimise interactions within their community. However, dung succession had no clear

distinction between guilds in the species collected during this investigation. It may be that competitive interactions in dung beetles are not always strong since competition is not the only significant factor determining the structure of the dung insect community (Hanski and Cambefort, 1991b). Additionally, a longer time period may be needed for observation of changes in the local dung beetle community and diurnal activity in order to assess their competitive and colonisation strategies, since a survey by Sabu *et al.* (2007) only showed a decline in beetle species and abundance after four days.

Various studies have yielded contrasting results with regard to comparisons in dung beetle diversity between reserves and agricultural landscapes. Similarities between reserves and agricultural landscapes were observed by Doube (1991) while other studies yielded differences in diversity between natural forests and agroecosytems (Estrada and Coates-Estrada, 2002; Avendano-Mendoza *et al.*, 2005; Harvey *et al.*, 2006). Further research needs to be carried out within the reserve as well as the surrounding agricultural landscape in order to ascertain patterns of distribution of dung beetle, their diversity and if any species could be used as indicators of either environment in Swaziland. The large scale modifications of the natural landscape may influence dung beetle distribution due to their close association with large mammals (Avendano-Mendoza *et al.*, 2005), which are absent outside the reserve. Maintenance of natural habitats, such as those found in reserves, is important for the maintenance of dung beetle species diversity. It has been documented that up to 72% of dung beetles species in South Africa and Namibia have their habitat protected by the existing nature reserve networks (Koch *et al.*, 2000). Due to their various roles in ecosystem function, an understanding of how invertebrate diversity may be protected is an important priority for conservation efforts. Mlawula Nature Reserve is thus important for maintenance of landscape heterogeneity, to promote overall diversity compared to the surrounding agricultural landscape. Preservation of optimal habitat and its quality within Mlawula and surrounding conservation areas such as Hlane and Mbuluzi Nature Reserve is imperative since these landscape fragments are critical to the dung beetle population for dispersal between these conservation fragments (Söderström *et al.*, 2001; Debano, 2006). Although observed at a comparatively smaller scale, evidence of movement of carabid beetles between habitat patches was observed within an agricultural landscape. These patches were also important for maximising coccinellid diversity within the same locality (Magagula and Samways, 2001; Magagula, 2003), thus emphasising the importance of natural habitats in modified landscapes. However, determination of the ideal distance between these reserves for the dung beetles is important for long term management and population viability (Koch *et al.*, 2000).

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# Morphological and quantitative studies of haemocytes in *Danaus chrysippus* Linn. (Lepidoptera: Nymphalidae)

**J. P. Pandey[1], R. K. Tiwari[2], Suyog Pandey[2] and Dinesh Kumar*[3]**

[1]*Department of Animal Sciences, School of Life Sciences, University of Hyderabad, Hyderabad 500 046, India*
[2]*Department of Zoology, K.N. Govt. Postgraduate College, Gyanpur 221 304, India*
[3]*Department of Zoology, Banaras Hindu University, Varanasi 221 005, India*
*Email: dines1953@yahoo.com*

ABSTRACT: Six main types of haemocytes, viz. prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs), adipohaemocytes (ADs) and oenocytoids (OEs) were observed in the blood of fifth instar larvae of *Danaus chrysippus*. In addition, vermicytes (VEs) regarded as a subtype, being the variant of PLs, were seen only in late fifth larval (prepupal) and early pupal stages. Presence of VEs appears to be important during the larval–pupal moult to phagocytose the debris of histolysed tissues. Total haemocytes counts (THC) increased during fifth instar larval development, reaching its maximum in prepupa. A gradual decline in their number occurred throughout the pupal stage. 

KEYWORDS: haemocytes, butterfly, *Danaus chrysippus*

The larvae of *Danaus chrysippus* Linn. (Lepidoptera: Nymphalidae) feed voraciously on leaves of *Calotropis gigantea* a shrub containing alkaloid. The alkaloid of *C. gigantea* is toxic to humans but *D. chrysippus* thrives well on the plant. It had been suggested that the haemocytes might play an important role in modifying the toxic effect of the host alkaloids (Russo *et al.*, 2001). The present study was undertaken to characterize and quantify the haemocytes of *D. chrysippus* larva.

The early larval instars of *D. chrysippus* Linn. were collected from *Calotropis gigantea* and reared in BOD incubator at 27 ± 1 °C, 75 ± 5% RH and 16L: 8D photophase. Immature stages of required age groups and newly emerged imagoes used in the present study were collected from the culture. Fifth instar larva 12 h before pupation was taken as prepupal stage.

Haemolymph from cut prolegs of larvae and punctured abdomen of pupae and adults was used. The mean number of the circulating haemocytes per $mm^3$ was

*Corresponding author

TABLE 1. Total haemocyte count of *Danaus chrysippus*

| Life stage | Count in different growth stages | | | | | | |
|---|---|---|---|---|---|---|---|
| Larva/ Pre-pupa | IV | $V_0$ | $V_{24}$ | $V_{48}$ | $V_{72}$ | $V_{96}$ | $V_{108}$(pp) |
| | 4870 ± 503 | 2285 ± 315 | 3250 ± 360 | 6510 ± 405 | 11208 ± 490 | 13345 ± 620 | 15290 ± 685 |
| Pupa/ adult | $P_0$ | $P_{24}$ | $P_{48}$ | $P_{72}$ | $P_{96}$ | P late | $A_0$ |
| | 3175 ± 340 | 2360 ± 320 | 1540 ± 135 | 915 ± 176 | 685 ± 60 | 205 ± 46 | 280 ± 60 |

IV, late fourth instar; V, fifth instar; pp, pre-pupa; P, pupa; A=adult.
Figures with each stage denote the age in hours. Values are mean ± SD for 20 insects.

calculated following the method of Jones (1962). Preparations of haemolymph smear, staining and calculation of percentage of different haemocytes were as described by Tiwari *et al.* (2006) and Pandey *et al.* (2008a,b).

Six types of blood cells were distinguished based on their morphological and cytological features. These were prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs), spherulocytes (SPs), adipohaemocytes (ADs) and oenocytoids (OEs). In addition, vermicytes (VEs) were seen in late fifth larval instar and early pupal instar.

The haemocytes present in the haemolymph of *D. chrysippus* are typical as found in other lepidopterans (Raina, 1976; Kurihara *et al.*, 1992; Ribeiro *et al.*, 1996; Sharma *et al.*, 2003; Falleiros *et al.*, 2003; Pandey *et al.*, 2008a,b) However, variations exist in earlier reports on haemocytes in the members of superfamily Nymphalidae. Takada and Kitano (1971) identified four types of haemocytes, namely prohaemocytes (PRs), plasmatocytes (PLs), granulocytes (GRs) and oenocytoids (OEs) in the fifth instar larvae of *Pieris rapae crucivora*. Salehi (1990) found six main types in *Papilio demoleus*: spherulocytes (SPs) and adipohaemocytes (ADs) being the two additional types. He further reported two more sub-types: podocytes (POs) and vermicytes (VEs) in 24 h old pupae. Pandey *et al.* (2003) reported all these cell types in *Papilio demoleus* with the difference that the VEs were seen in both pre-pupae and newly formed pupae while POs were seen only in 0 h old pupae. The cell types in *D. chrysippus* are similar to those found in *P. demoleus*.

There is no agreement on the existence of VEs as a distinct cell type. While some give separate identity (Kurihara *et al.*, 1992; Ribeiro *et al.*, 1996; Falleiros *et al.*, 2003; de Andrade *et al.*, 2003), others (Pandey *et al.*, 2003) consider them as a variant of PLs. In *D. chrysippus*, the VEs can be regarded as modified PLs because they are similar in shape and appear in prepupal and pupal stages only. Their presence in larval-pupal stage suggests they are associated with the phagocytosis of the debris of the histolysed tissues (Gupta, 1985).

A similar situation exists for ADs too. In some insects (Salehi, 1990; Pandey *et al.*, 2003), these cells were described as distinct cell types while in other studies (Takada and Kitano, 1971; Arnold, 1982; Sharma *et al.*, 2003; Gelbic *et al.*, 2006) they were not detected. Raina (1976) described ADs as mature stage of GRs after lipid incorporation. Recently, Falleiros *et al.* (2003) also reported that ADs showed ultrastructural similarity with GRs, with large number of lipid droplets. This could be

TABLE 2. Differential count of haemocytes of *Danaus chrysippus*

| Haemocyte Type | Count in different growth stages | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| PR | IV late | $V_0$ | $V_{24}$ | $V_{48}$ | $V_{72}$ | $V_{96}$ | $V_{108}$(pp) | $P_0$ |
| | 12.7 ± 1.0 | 11.27 ± 1.4 | 10.2 ± 0.6 | 4.8 ± 0.7 | 4.6 ± 0.6 | 4.5 ± 0.3 | 4.2 ± 1.1 | 3.4 ± 0.4 |
| PL | 37.2 ± 2.1 | 21.7 ± 2.0 | 23.6 ± 1.2 | 29.8 ± 2.6 | 30.5 ± 1.2 | 32.1 ± 2.8 | 37.5 ± 2.3 | 19.6 ± 2.4 |
| GR | 27.3 ± 2.7 | 26.9 ± 0.7 | 25.9 ± 1.6 | 24.7 ± 1.9 | 23.1 ± 0.9 | 27.2 ± 1.0 | 20.1 ± 1.2 | 17.7 ± 3.2 |
| SP | 11.3 ± 1.9 | 33.4 ± 3.1 | 26.6 ± 2.3 | 24.5 ± 1.2 | 20.3 ± 1.3 | 14.4 ± 2.2 | 13.5 ± 2.0 | 12.1 ± 0.8 |
| AD | 7.6 ± 1.3 | 3.7 ± 1.0 | 10.8 ± 1.3 | 12.5 ± 0.8 | 13.2 ± 1.2 | 13.1 ± 1.1 | 14.7 ± 1.3 | 24.9 ± 2.8 |
| OE | 3.9 ± 0.4 | 3.1 ± 0.2 | 2.9 ± 0.2 | 3.7 ± 0.4 | 8.3 ± 1.1 | 8.7 ± 0.5 | 8.2 ± 0.7 | 12.9 ± 1.3 |
| VE | – | – | – | – | – | – | 1.8 ± 0.5 | 9.4 ± 1.4 |

IV, late fourth instar; V, fifth instar; pp, pre-pupa; P, pupa; A, adult.
Figures with each stage denote the age in hours. Values are mean ± SD for 20 insects.

one of the reasons for higher percentage of GRs in those lepidopterans wherein ADs are not detected.

The THC pattern (Table 1) with a continuous increase during fifth instar larval development and a sudden decrease in 0 h old pupae of *D. chrysippus* is similar to the observations in *P. demoleus* (Tiwari and Shukla, 2000) and differs from the decreasing trend seen in *Anticarsia gemmatalis* (de Andrade *et al.*, 2003).

The DHC (Table 2) in *D. chrysippus* exhibited similarity with majority of lepidopterans (Gupta, 1979; Bombonato and Gregorio, 1995; Falleiros *et al.*, 2003), wherein PRs, OEs and VEs show lower frequency. PLs and GRs exhibit high frequencies. This is in contrast to the report of de Andrade *et al.* (2003) who in *A. gemmatalis* larvae reported 0.9% PRs and 49.7% PLs.

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# Changes in protein profile of *Apis mellifera* L. (Hymenoptera: Apiidae) worker haemolymph after exposure to cell phone radiation

**Neelima R. Kumar, Sonika Sangwan, Pooja Badotra* and V. P. Sharma**

*Department of Zoology, Panjab University, Chandigarh 160 014, India*
*Email: neelimark@yahoo.co.in*
*Email: pooja_badotra@yahoo.co.in*

ABSTRACT: We studied the impact of cell phone radiations on biochemical parameters of haemolymph of workers of *A. mellifera*. Protein concentration in the control sample was 0.475 mg/ml. In the treated sample the protein concentration increased significantly on 10 min and 20 min exposure, with a slight decrease on 40 min exposure. Three free amino acids L-Cystine, Histidine and Hydroxyproline were indentified in the haemolymph of control. In case of 10 min exposure D-Serine was present instead of Hydroxyproline and a fourth amino acid present could not be identified. In the 20 and 40 min exposure, two additional amino acids DL-Ornithine and DL-Arginine were identified. SDS-PAGE showed 14 protein bands in control as compared to 16 after 10 min, 17 after 20 min and 15 after 40 min exposure to EMR from cell phones. 

KEYWORDS: cell phone radiation, *Apis mellifera*, haemolymph proteins

Today's environment has a high level of electro-pollution. The man-made electromagnetic fields now blanket the earth because of a vast network of systems like mobile telephony. EMR and phone networks have been implicated in behavioural, biological and biochemical changes in birds, mammals and honey bees (Lai and Singh, 1995, 1996; Balmori, 2003; Everaert and Bauwens, 2007).

Honey bees are known for their navigation skills. They orient themselves according to the earth's magnetic field and possess magnetite in their abdomen which helps in this orientation mechanism. It is only to be expected therefore that the behaviour of bees will be influenced in one way or another by electromagnetic radiations. Stever and Kuhn (2001) and Harst *et al.* (2006) reported reduction in returning ability of honey bees exposed to cell phone radiations from Digital Enhanced Cordless Telecommunication Telephone. The growth of the colony measured in terms of bee

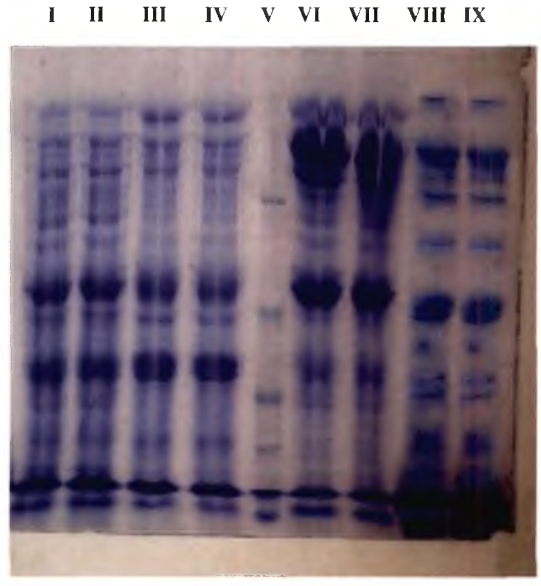
*Corresponding author

TABLE 1. Protein types in the haemolymph of *Apis mellifera* before and after exposure to cell phone radiation

| Molecular weight (kD) of proteins on exposure for periods | | | |
|---|---|---|---|
| Nil (Control) | 10 min | 20 min | 40 min |
| 192.8 | — | — | — |
| 164.1 | — | — | — |
| 153.1 | — | — | — |
| — | 146.6 | 146.2 | 146.2 |
| 139.6 | — | — | — |
| 136.5 | — | 136.5 | 136.5 |
| — | 128.8 | 128.8 | — |
| — | 124.5 | 124.9 | 124.5 |
| — | 110.9 | 110.5 | 110.9 |
| — | 103.5 | 103.5 | 103.5 |
| — | — | 98.86 | — |
| — | 90.16 | 90.26 | 90.1 |
| — | 81.14 | 81.14 | — |
| 80.35 | — | — | 80.52 |
| 70.86 | 70.15 | — | 70.99 |
| — | — | 69.98 | — |
| — | — | — | 68.39 |
| 67.31 | 67.61 | 67.61 | — |
| 60.73 | 60.95 | 60.95 | 60.21 |
| 48.98 | 48.98 | 48.98 | — |
| — | — | — | 47.32 |
| 42.66 | — | — | — |
| 39.36 | — | — | — |
| — | 38.02 | 38.02 | 38.02 |
| — | 36.31 | — | — |
| — | — | 35.48 | — |
| — | — | — | — |
| — | 33.11 | 33.11 | 33.11 |
| — | 30.2 | — | 30.9 |
| 28.1 | — | 28.18 | 28.11 |
| — | 27.54 | — | — |
| 26.3 | — | — | — |

strength, brood area, queen prolificacy and nectar and pollen stores has also been observed to be significantly less in the exposed colonies (V. S. Sharma 2009, pers. comm.). The present study was aimed at understanding the physiological changes in haemolymph proteins in *Apis mellifera* (Hymenoptera: Apiidae) workers exposed to cell phone radiations.

Samples of *Apis mellifera* adult workers were drawn from the colonies maintained by the Department of Zoology, Panjab University, Chandigarh. Observation hives with wire gauze fixed at the sides were used for the experiment. Mobile phones used were




Lane I, II : 40 min exposure sample

Lane III, IV : 20 min exposure sample

Lane V : Standards

Lane VI, VII : 10 min exposure sample

Lane VIII, IX : Control sample

FIGURE 1. SDS-PAGE of *A. mellifera* worker.

of the same make and model and operated on the same network. Two mobile phones each were placed against the wire gauze side of one bee hive and another hive was kept without mobile phones (control). Phones were kept in listen — talk mode for 40 min using a tape recorder. Twenty honey bees were collected from the exposed and control hives at intervals of 10, 20 and 40 min.

Haemolymph of worker bees was collected with a micropipette inserted into the intersegmental region of the bee's abdomen. Equal volume of the haemolymph from all bees was dissolved in 1 ml of normal saline or PBS as the experiment demanded.

Protein was estimated by Lowry's method (Lowry *et al.*, 1951). Qualitative assay of haemolymph proteins was done by SDS-PAGE (Laemmli, 1971). Free amino acids were studied by thin layer chromatography.

In the haemolymph of control bees the protein concentration was $0.475 \pm 0.002$ mg/ml. In treated bees, the protein concentration was $0.525 \pm 0.003$ mg/ml after 10 min exposure, $0.825 \pm 0.002$ mg/ml after 20 min exposure and $0.650 \pm 0.003$ mg/ml after 40 min exposure. The differences were significant at $p < 0.005$.

TABLE 2. Free amino acids identified in control and treated haemolymph samples of *Apis mellifera* adult worker

| Control | Exposed to cell phone radiation for | | |
|---|---|---|---|
| | 10 min | 20 min | 40 min |
| — | unknown | — | — |
| — | — | DL-Ornithine | DL-Ornithine |
| L-Cystine | L-Cystine | L-Cystine | L-Cystine |
| Histidine | Histidine | Histidine | Histidine |
| Hydroxyproline | — | — | — |
| — | D-Serine | D-Serine | D-Serine |
| — | — | DL-Alanine | DL-Arginine |

In SDS-PAGE analysis, 14 bands corresponding to protein types with different molecular weights were observed in the haemolymph of control sample (Table 1 and Fig. 1). The number of protein bands in bees exposed to cell phones were 16 for 10 min exposure, 17 for 20 min and 15 for 40 min.

The chromatogram showed three spots for free amino acids in the haemolymph of non-exposed workers while there were four, five and five in case of 10, 20 and 40 min exposure, respectively (Table 2).

Very little work has been done on biochemical, metabolic and physiological influence of cell phone radiation in animals. The present study showed an increase in haemolymph protein concentration in 10 min and 20 min exposed samples. The concentration decreased after 40 min exposure but still remained higher than in the control. The number of protein types also showed a similar increase and decrease. Bindokas *et al.* (1998) studied the influence of 765 kV transmission lines on the circulating haemocytes and blood protein pattern of adult worker bees and found no consistent effect on mean haemocyte counts or slab gel electrophorograms. This is unlike the present results probably because of difference in the nature of field tested and the technique used. In the present study an increase was also recorded in the number of free amino acids in the haemolymph of worker *A. mellifera* on exposure to cell phone radiations. It has previously been reported that the concentration of all amino acids in the haemolymph of *A. mellifera* prepupae increased when they were exposed to X-rays (Richardson and Myser, 1973). According to these workers this increase was due to impairment of protein synthesis in the tissues and consequent accumulation of the available amino acids. The same could be true for the present observation suggesting that cell phone radiations interfered with normal metabolic processes increasing the concentration of biomolecules to abnormal levels which could then influence the behaviour of bees.

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# *Phenococcus solenopsis* Tinsky (Hemiptera: Pseudococcidae) as a major pest of Bt-cotton in Warangal, Andhra Pradesh

**P. Laxman, Ch. Sravanthy, A. Nageswara Rao and Chintha Sammaiah***

*Environmental Biology Research Lab, Department of Zoology Kakatiya University, Warangal, Andhra Pradesh, India*
*Email: sammaiah_ch@yahoo.com*
*Email: peesarilaxman@yahoo.in*

ABSTRACT: The mealy bug, *Phenococcus solenopsis* Tinsky was recorded as a major pest of Bt-cotton in agricultural fields in and around Warangal, Andhra Pradesh, India. The percentage of infested plants ranged from 47 to 53 during September to December in 2007 and 2008. Many severely infested plants were found dead.


KEYWORDS: *Phenococcus solenopsis* Tinsky, mealy bug, Bt-cotton

Bt-cotton is an important crop cultivated in Telangana, the semi-arid region of Warangal, Andhra Pradesh, India. We report here the occurrence of the mealy bug, *Phenococcus solenopsis* Tinsky (Hemiptera: Pseudococcidae) as a serious pest of Bt-cotton. This insect has earlier been reported attacking a variety of cultivated crops such as tomato, brinjal, chilli, and the ornamental plant *Hibiscus rosasmensis* in Bihar, Delhi, Karnataka and Maharashtra (Hayat, 1986; Sinha *et al.*, 1985). Among mealy bugs, only *Ferrisia virgata* CKII. has been reported earlier as a major pest of cotton, from Pakistan (Ghouri, 1960) although it has been recorded from other malvaceous genera like *Abutilon*, *Hibiscus* and *Malvastrum* (Ben-Dov, 1994). *P. solenopsis* appeared on Bt-cotton during the year 2005 and attained the status of a serious pest in Bt-cotton growing areas of Warangal, Adilabad, Karimnagar and Khammam (Dileep Kumar *et al.*, 2008).

We carried out a survey by "fixed plot method" (Govindaiah and Gunashekar, 1992) in five Bt-cotton fields with similar crop pattern in agricultural fields surrounding Warangal. The survey was made during June to December in the years 2007 and 2008. In each selected cotton field, five plots of 5 m × 5 m were marked out (one each in the four corners, 10 m away from the border, and one in the centre), thus making a total of

*Corresponding author

FIGURE 1. Bt-cotton plant dying due to attack of *P. solenopsis*.

TABLE 1. Infestation of Bt-cotton plants by *Phenococcus solenopsis*

| Month of observation | Percentage of plants attacked | |
|---|---|---|
| | Year 2007 | Year 2008 |
| July | Nil | Nil |
| August | Nil | Nil |
| September | 36.72 ± 6.79 | 36.76 ± 3.28 |
| October | 40.10 ± 7.80 | 41.81 ± 9.92 |
| November | 32.30 ± 7.90 | 53.1 ± 6.98 |
| December | 53.24 ± 3.07 | 50.72 ± 5.35 |

25 plots. In each plot the total number of plants and the number of mealy bug infested plants were counted and the percentage of infestation was worked out. Observations were recorded at monthly intervals.

No infestation was recorded during the first eight weeks of the crop. Infestation by *P. solenopsis* on the stem and leaves of the cotton plants at the apical shoots was noticed first during the tenth week and by 21st week the crop was heavily infested. Some of the severely infested plants died. (Fig. 1)

Table 1 shows the month wise percentage of infestation. It may be seen that the percentage of infested plants ranged from 47 to 53 during September to December in 2007 and 2008. Many severely infested plants were found dead.

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# Insecticidal activity of certain plant extracts against pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae)

**S. R. Yankanchi*, S. A. Koli and P. A. Patil**

*Department of Zoology, Shivaji University, Kolhapur 416 004, , Maharashtra, India*
*Email: sryankanchi@yahoo.co.in*

ABSTRACT: Leaf extracts of the plants, *Vitex negundo*, *Withania somnifera*, *Tridax procumbens* and *Cassia tora* were screened against *Callosobruchus chinensis* at concentrations of 0.12 mg, 0.25 mg and 0.5 mg/g seed. Extract of *V. negundo* and *W. somnifera* at 0.5% were more effective, causing 100% adult mortality and progeny suppression. *T. procumbens* and *C. tora* extracts showed less oviposition deterrence compared to the other two. 

KEYWORDS: botanicals, *C. chinensis*, progeny suppression, oviposition deterrence

The pulse beetle, *Callosobruchus chinensis* L. (Coleoptera: Bruchidae) is the most widespread and major insect pest of stored pulses (Park *et al.*, 2003). Organophosphates and fumigants are still effective means of protection of stored legumes and other agricultural commodities from insect infestation (EPA, 2001). Although effective, the use of insecticides causes environmental problems and hence the need for alternative materials arose. A number of plant species have been found effective against stored grain pests (Dubey *et al.*, 2008; Yankanchi and Gonugade, 2009). Therefore four medicinal plants viz. *Vitex negundo*, *Withania somnifera*, *Tridax procumbens* and *Cassia tora* were evaluated for the control of *C. chinensis*.

The adults of *C. chinensis* were reared in the laboratory under constant temperature (28 ± 2 °C) and relative humidity (70 ± 5%). A common method developed for extraction from the dried plant materials was followed (Pavela *et al.*, 2008). Dry powder material (25 g) was extracted with 250 ml methanol in Soxhlet apparatus for 10 h. The solvent was removed from the extract using a vacuum evaporator. Three doses of 2.5, 5 and 10 mg dried residue was dissolved in 1 ml analytical grade acetone and was tumble mixed with 20 g green gram seeds in plastic containers and air-dried. Seeds treated with 1 ml acetone alone were kept as control. Ten pairs of 0–24 h old adult bruchids were introduced to each container and mortality was observed after

*Corresponding author

TABLE 1. Effect of plant extracts on mortality, oviposition and suppression of $F_1$ progeny of *C. chinensis*

| Plant extract (mg/g seeds) | Corrected mortality (%) | Oviposition deterrence (%) | $F_1$ Progeny suppression (%) |
|---|---|---|---|
| *V. negundo* | | | |
| 0.12 | $67.8^{b}$ | $55.1^{c}$ | $100^{a}$ |
| 0.25 | $78.4^{b}$ | $81.2^{a}$ | $100^{a}$ |
| 0.5 | $100^{a}$ | $92.6^{a}$ | $100^{a}$ |
| *W. somnifera* | | | |
| 0.12 | $73.1^{b}$ | $73.3^{b}$ | $100^{a}$ |
| 0.25 | $87.5^{a}$ | $86.4^{a}$ | $100^{a}$ |
| 0.5 | $100^{a}$ | $96.8^{a}$ | $100^{a}$ |
| *T. procumbens* | | | |
| 0.12 | $24.9^{c}$ | $44.1^{c}$ | $48.7^{c}$ |
| 0.25 | $42.2^{c}$ | $57.3^{c}$ | $74.7^{b}$ |
| 0.5 | $73.1^{b}$ | $68.0^{b}$ | $100^{a}$ |
| *C. tora* | | | |
| 0.12 | $28.8^{c}$ | $23.0^{d}$ | $56.7^{c}$ |
| 0.25 | $32.7^{c}$ | $36.3^{d}$ | $68.0^{b}$ |
| 0.5 | $69.2^{b}$ | $67.8^{b}$ | $100^{a}$ |
| Control 0 | $02.6^{d}$ | $08.6^{d}$ | $11.2^{d}$ |

Means followed by same letters within each column do not differ significantly in DMRT ($p < 0.05$).

24 h of exposure and were corrected following Abbott (1925). The total number of eggs laid on the seed was recorded after 5 days of treatment and percent oviposition deterrence was calculated according to Elhag (2000). To determine the $F_1$ progeny suppression of plant extracts, 20 g green gram seeds (each seed with an egg) were placed in separate plastic containers and treated with the above doses of extracts. After 25 days, the number of adults emerged was recorded and percent suppression was calculated following Aldryhim (1995). Treatments were replicated thrice. Data were subjected to one-way ANOVA. Means were compared using Duncan's multiple range test (DMRT).

Data (Table 1) showed that 0.5% extracts of *V. negundo* and *W. somnifera* gave 100% kill of *C. chinensis* adults and was the most toxic. But 0.25% extract of *W. somnifera* giving 87.5% kill of adults also came on par with the same. Extracts (0.5%) of *T. procumbens* and *C. tora* giving 73.1% and 69.2% kill came on par and significantly inferior to *V. negundo* and *W. somnifera* at the same doses but were on par with 0.25% and 0.12% of *V. negundo* and 0.12% *W. somnifera*. At the concentrations of 0.25% and 0.12% the mortality in *T. procumbens* and *C. tora* extracts were on par and least effective. Thus results show that *W. somnifera* and *V. negundo* were most toxic to *C. chinensis*. The other two plant extracts were on par and inferior to *V. negundo* and *W. somnifera* with reference to their toxicity to *C. chinensis* adults.

With reference to oviposition deterrence *W. somnifera* came on par with *V. negundo* at the higher two doses and at 0.12% concentration the former was significantly inferior to the latter. *T. procumbens* and *C. tora* (68.0% and 67.8% deterrence, respectively) came on par with 0.12% of *W. somnifera* extract and was even better than 0.12% of *V. negundo* extract which gave 55.1% deterrence only. With reference to the suppression of $F_1$ progeny production all the doses of *V. negundo* and *W. somnifera* and highest dose of *T. procumbens* and *C. tora* came on par. Remaining treatments were significantly inferior to the former extracts.

Leaf extracts of *V. negundo*, *W. somnifera*, *T. procumbens* and *C. tora* showed decrease in grain damage, indicating the presence of toxic components in these plants. Chemical components of *V. negundo* leaf are flavonoids, glycoside, lignans, and terpenes (Yankanchi, 2009). *W. somnifera* leaf has alkaloids viz. solanine, solanidine, nicotine, somniferine, somnifernine, somnine, withananine, withanine, withananinine, volatile oil and tannin (Khoshnoud *et al.*, 2008) which might be toxic to *C. chinensis*. Gupta and Srivastava (2008) using plant parts of *W. somnifera* against *C. chinensis* showed that leaf extract was most effective in causing adult mortality. Furthermore essential oils of plants were used to control stored product pests (Keita *et al.*, 2001; Kim *et al.*, 2003; Park *et al.*, 2003). Essential oils can not be applied to control infestation of food commodities stored in jute bags because of the loss due to volatility (Shukla *et al.*, 2009).

All extracts at 0.5% suppressed progeny production fully, thus indicating that even if oviposition occurred before death, the activity of extracts on larval development was adequate to control the pest.

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ENTOMON **34(4)**: 267–268 (2009)
Short Communication No. ent.34409

# Incidence of *Lema downsei* Baly (Coleoptera: Chrysomelidae) on *Asparagus racemosus*

**Vipin Chaudhary and L. Saravanan**

*Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand, Gujarat, India*
*Email: vipin_cima@yahoo.com*

ABSTRACT: *Lema downsei* (Coleoptera: Chrysomelidae) is recorded as a new pest of *Asparagus racemosus*. 

KEYWORDS: *Lema downsei*, *Asparagus racemosus*, new record

The chrysomelid beetle, *Lema downsei* Baly is reported to infest wheat (David and Ananthakrishnan, 2006). The occurrence of this beetle was noticed on *Asparagus racemosus* in the herbal garden of the Directorate of Medicinal and Aromatic Plants Research at Boriavi, Anand, Gujarat, India. *A. racemosus* is an under-shrub that climbs up to 1–3 m high, with stout and creeping root stock. It is found in plains to 1200 m high, in tropical and sub-tropical, dry and deciduous forests and in the Himalayas. The infestation was noticed in February 2009, during regular surveys for insect pests on medicinal plants. Both the adults and grubs in particular were found chewing the tender tips of new shoots and tender foliage which resulted in shallow holes on the shoot and brownish discolouration of the tissues. The affected tender shoots ultimately dried out. On an average 10-12 grubs and 5-6 adults per shoot were recorded whenever there were new shoots. The adults were active throughout the year except during the winter months. They chewed on the foliage in the absence of tender new shoots, causing drying of leaves. Heavy infestation could weaken the plants and reduce the plant's root yield. Other pests reported on *A. racemosus* are grasshoppers, aphids, army worms and a mite but no major damage is observed (Gupta, 2005).

This is the first report of the *Lema downsei* on *A. racemosus* from India.

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# TNAU egg removal device in the management of insect pests of stored Siddha and Ayurvedic ingredients

**S. Divya and S. Mohan***

*Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore 641 003, India*
*Email: sarmaprashanth@rediffmail.com*
*Email: divyaento@rediffmail.com*

ABSTRACT: An egg removal device (Indian Patent No. 198434) tested for the control of insect pests attacking stored ingredients of Siddha and Ayurvedic medicines was found effective, resulting in significantly lower offspring production in the treatment compared to control. 

KEYWORDS: insect egg removal, stored products, Siddha and Ayurvedic medicines

Insect pests are found to infest various stored commodities of medicinal value used in the preparation of Siddha and Ayurvedic medicines. Among them, *Lasioderma serricorne* (Fab.), *Oryzaephilus surinamensis* (L.), *Tribolium castaneum* (Herbst.) and *Tenebroides mauritanicus* (L.) cause serious damage and reduce the quality of the product. Using pesticides for control of such stored product insects is not recommended due to its adverse effect on human health and export potential of the products. There is an increasing need for finding safer methods for the management of stored product insects attacking the ingredients of Siddha and Ayurvedic medicines. Since an egg removal device developed in Tamil Nadu Agricultural University, Coimbatore (Indian Patent No. 198434) has been found effective in crushing the eggs of *Callosobruchus* sp. glued to the seed coat without causing any damage to seeds it was tested for the above purpose.

The TNAU egg removal device has been described by Mohan (2005). The commodities selected were turkey berry fruits, calotropis flowers and valvuluvai rice seeds which are commonly used for non-allopathic systems of treatment. One kilogram each of the above infested commodities were collected from a private firm and brought to the laboratory. Thirteen such samples randomly collected were used for the study. Half of each sample was kept as control and the other half was treated in the egg removal

*Corresponding author

TABLE 1. Efficiency of egg removal device in the control of stored product insects in tested commodities

| Commodity | Category | Mean no. of adults of *O. surinamensis* at initial stage | Mean no. of life stages of *Oryzaephilus surinamensis* at 60 DAS | | | | Mean no. of adults of *L. serricorne* at initial stage | Mean no. of life stages of *Lasioderma serricorne* at 60 DAS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Egg | Larva | Pupa | Adult | | Egg | Larva | Pupa | Adult |
| Turkey berry fruits | Treated | 0.2 (0.79) | 0.4 (0.91) | 0.4 (0.91) | 0.2 (0.83) | 0.4 (0.91) | 1.2 (1.28) | 0.4 (0.91) | 0.4 (0.91) | 0.2 (0.83) | 1.1 (1.23) |
| | Control | 0.2 (0.79) | 1.3 (1.32) | 1.4 (1.34) | 1.7 (1.46) | 1.5 (1.37) | 1.5 (1.39) | 1.8 (1.49) | 1.2 (1.28) | 0.4 (0.91) | 3.2 (1.9) |
| | CD ($P = 0.05$) | 0.1259 | 0.1732 | 0.1874 | 0.1653 | 0.1891 | 0.1757 | 0.1726 | 0.2009 | 0.1909 | 0.1880 |
| Calotropis flowers | Treated | 0.6 (1.03) | 1.2 (1.28) | 1.1 (1.23) | 0.6 (1.03) | 1.4 (1.36) | 1.2 (1.28) | 1.8 (1.47) | 1.4 (1.33) | 0.8 (1.08) | 1.9 (1.54) |
| | Control | 0.9 (1.03) | 2.6 (1.76) | 2.4 (1.68) | 1.7 (1.46) | 2.2 (1.61) | 1.1 (1.23) | 3.5 (2.01) | 2.7 (1.78) | 2.2 (1.61) | 5.8 (2.5) |
| | CD ($P = 0.05$) | 0.1698 | 0.1183 | 0.1784 | 0.1759 | 0.1712 | 0.1989 | 0.1829 | 0.2020 | 0.2020 | 0.1738 |
| Vaalvuluvai rice seeds | Treated | 1.5 (1.42) | 2.2 (1.64) | 1.4 (1.36) | 1.1 (1.23) | 1.7 (1.46) | 1.2 (1.27) | 0.5 (0.99) | 0.6 (1.00) | 0.4 (0.91) | 0.6 (1.03) |
| | Control | 1.7 (1.46) | 3.2 (1.93) | 2.5 (1.71) | 1.8 (1.51) | 3.5 (1.99) | 1.2 (1.25) | 2.3 (1.66) | 1.4 (1.34) | 1.2 (1.24) | 2.8 (1.82) |
| | CD ($P = 0.05$) | 0.1595 | 0.1334 | 0.1217 | 0.1972 | 0.1916 | 0.1798 | 0.1832 | 0.1798 | 0.1652 | 0.1511 |

DAS, Days After Storing; Values are the mean of 13 observations. Figures in parentheses are square root (X+0.5) transformed values.

device. Each sample was treated for two hours continuously for three days and then stored in normal containers for a period of 60 days, as generally it takes 50–60 days for completing one generation in stored product insects. The treatment was repeated 13 times with half kilogram of sample for each replication and half kilogram of sample as control. The number of eggs, larvae, pupae and adults were counted on 60th day after storage.

All the life stages of *O. surinamensis* and *L. serricorne* were significantly lower in treatments compared to corresponding control (Table 1). The variations in the population on 60th day after storage can be attributed to the physical disturbances received by the life stages of insects while treating them in the device. Loschiavo (1978) observed that increase in physical impact lead to increase in the mortality of larvae of stored product insects. Quentin *et al.* (1991) observed that periodical tumbling of dry kidney bean (*Phaselous vulgaris* L.) controlled the infesting larvae of *Acanthoscelides obtectus* (Say). The TNAU egg removal device would prove to be very effective in the control of insect pests of stored ingredients used for the preparation of Siddha and Ayurvedic medicines.

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# Errata

This figure replaces FIGURE 1 printed on PAGE 202 of ENTOMON 34(3) and forms part of the paper '*Cleonaria bicolor* Thomson (Coleoptera: Cerambycidae): a new pest of *Ixora*' authored by K.D. Prathapan, M.H. Faizal and K.N. Anith

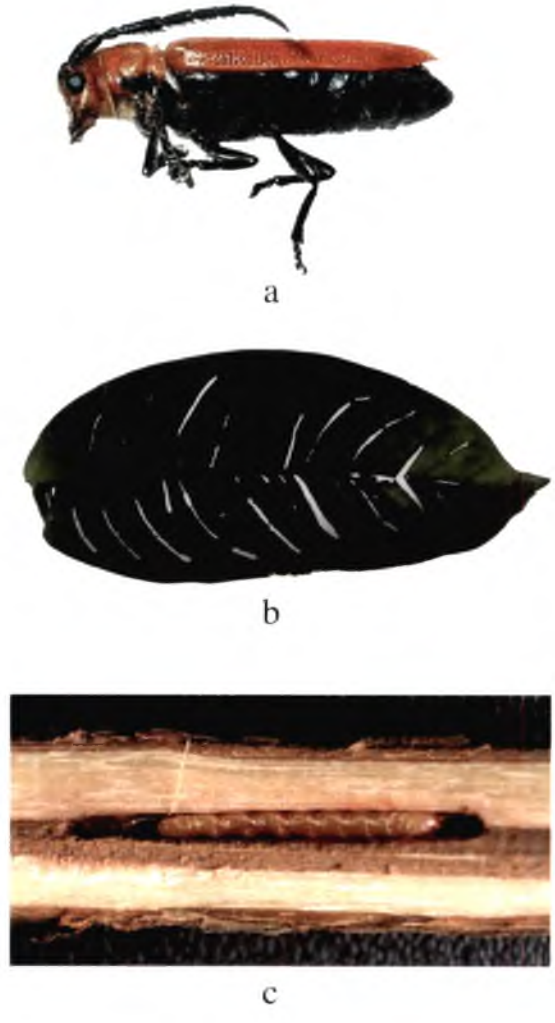


FIGURE 1. *Cleonaria bicolor*: a, adult; b, adult damage on leaf; c, larva within the stem.

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Jalaja M., Muraleedharan D. and Prabhu V. K. K. (1973) Effect of extirpation of median neurosecretory cells on reproduction in the female red cotton bug, *Dysdercus cingulatus*. Journal of Insect Physiology, 19(1): 29–36.

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SHORT COMMUNICATIONS



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